

## ABSTRACTS

## Wladimir Adlivankine European Society of Endodontology Research Prize

## RP1

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**SEM-analysis of the integrity of resected root apices of cadaver and extracted teeth after ultrasonic root-end preparation at different intensities**

**Aim** To compare the integrity of root apices of cadaver and extracted teeth after resection, ultrasonic root-end cavity preparation at medium and low ultrasonic power settings and following extraction.

**Methodology** Root canal treatment, perpendicular root-end resection and root-end cavity preparation were performed on singlerooted anterior and premolar teeth [49 teeth *in situ* in maxillary and mandibular jaws from cadavers (CT) and 45 extracted teeth (ET)]. Apical root-end cavities were prepared with the S12/90°D tip and the Suni-Max ultrasonic unit (Satelec, France) at the intensity prescribed by the manufacturer (Power 7 at power mode S) (34 CT, 30 ET) and at a lower intensity (Power 4 at power mode S) (15 CT, 15 ET). After ultrasonic preparation the cadaver teeth were retrieved from the jaws. Exaflex impressions (GC Corporation, Japan) were made of the root apices after resection, root-end preparation and retrieval. These impressions were processed for SEM-analysis, and the recordings evaluated for cracks and marginal chipping.

**Results** In general, extracted teeth showed significantly more cracks and chipping than cadaver teeth. Lowering the ultrasonic power from medium to low intensity resulted in equal scores for cracks on extracted teeth and for chipping on cadaver teeth, in higher scores for cracks on cadavers and in lower scores for chipping on extracted teeth. Complete cracks and cracks originating from the root surface occurred only in extracted teeth.

**Conclusions** The number of cracks and chipping caused by ultrasonic root-end preparation was higher on extracted teeth than on cadaver teeth. Lowering the ultrasonic power from medium to low intensity cannot be recommended as it resulted in more cracks and equal chipping on cadaver teeth. Investigation of techniques and materials should be conducted *in situ* and not on extracted teeth.

## RP2

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**Effectiveness of three different nickel-titanium rotary instruments for removing gutta-percha in curved root canals *in vitro***

**Aim** To compare *in vitro* the effectiveness of FlexMaster (Vereinigte Dentalwerke, Germany), ProTaper (Dentsply Maillefer, Switzerland), RaCe (FKG Dentaire, Switzerland) rotary instruments and Hedström hand files for removing gutta-percha in curved root canals during retreatment.

**Methodology** Sixty mandibular premolars with one single canal with a curvature between 20 and 36 degrees, determined by Schneider's method, were selected. The canals were enlarged to size 30 with FlexMaster instruments and filled using the cold lateral condensation technique. The teeth were randomly divided into four groups of 15 specimens each. After repretreatment with Gates Glidden burs and the test instruments the specimens were cleared. The area of remaining filling material on the root canal wall was measured from buccolingual and mesiodistal directions using a microscope and a computer image analysis program. Statistical analysis was performed using the Kruskal-Wallis test and a closed test procedure.

**Results** RaCe instruments revealed the least residual filling material. They removed significantly more gutta-percha and sealer than FlexMaster files ( $P < 0.05$ ). No significant difference was found among the Hedström, the ProTaper and the FlexMaster groups ( $P > 0.05$ ). The shortest time for removal of gutta-percha was observed with Hedström files ( $P < 0.05$ ). There were five fractured instruments in the ProTaper group, three in the FlexMaster group and none in the other groups.

**Conclusions** The RaCe rotary system was efficient and safe but time-consuming for gutta-percha removal in curved root canals. During retreatment the risk of instrument fractures of ProTaper and FlexMaster instruments was higher than that of RaCe and Hedström files.

**Acknowledgements** The authors thank Professor J. Schulte Mönning for his help with the statistical analysis and Vereinigte Dentalwerke, FKG Dentaire, and Dentsply Maillefer for the donation of the rotary instruments.

## RP3

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### ***In vitro* characterisation of the dentinogenic potential of Mineral Trioxide Aggregate**

**Aim** To provide an explanation for the biological effects of Mineral Trioxide Aggregate in vital pulp therapy.

**Methodology** Mouse odontoblast-like cells (MDPC-23) were grown on 24 hr set Mineral Trioxide Aggregate (MTA) for 24 hours and examined by scanning electron microscopy (SEM) for biocompatibility. Samples of normal human dentine were extracted with EDTA (10%), calcium hydroxide (0.02 M) and the soluble products of MTA (1.72 g in 1000 ml of water). The resulting extracellular dentine matrix protein preparations (E-DMP's) were analysed using 1D Polyacrylamide Gel Electrophoresis. A sandwich ELISA technique was employed to determine the concentration of TGF- $\beta$ 1 in each matrix preparation. MDPC-23 cells were exposed to concentrations of 1, 100, 1000 mg/mL of these matrix preparations for 24 h and analysed by reverse transcription – polymerase chain reaction (RT-PCR) to determine expression of TGF- $\beta$ 1.

**Results** MDPC-23 cells were observed to grow well on MTA over the 24 h culture period. MTA solubilised E-DMP's throughout the experimental period of 14 days whereas the action of calcium hydroxide was minimal after day 7. The protein profile identified by 1D PAGE analysis demonstrated a more diverse profile for E-DMP's solubilised with MTA. ELISA showed a higher concentration of TGF- $\beta$ 1 in E-DMP'S extracted with soluble products of MTA than calcium hydroxide. MTA solubilised E-DMP's caused significant up-regulation in gene expression for TGF- $\beta$ 1.

**Conclusions** MTA is capable of solubilising a heterogeneous profile of matrix components from human dentine over extended periods of time. The matrix components solubilised included the growth factor TGF- $\beta$ 1 and demonstrated bioactive properties on regulation of gene expression in odontoblast-like cells. The interactions of MTA with the cells and matrix in the dentine-pulp complex may complement its biocompatible properties and contribute to its biological effects on dentine bridge formation and regeneration in these tissues.

**Acknowledgements** This study was supported by a research grant from the Royal College of Surgeons of Edinburgh. The authors would like to thank Drs J Nor and C T Hanks, University of Michigan, USA, for the kind gift of the MDPC-23 cell line.

## RP4

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### **Antibacterial effects of different root-end filling materials**

**Aim** To evaluate the antibacterial activity of leachable components of amalgam, ProRoot MTA (Dentsply Maillefer, Switzerland), IRM (Caulk Dentsply, USA), Super Bond C&B (Sun Medical, Japan), Geristore (Den-Mat, USA), Dyract (Dentsply DeTrey, Germany) SE Bond (Kuraray, Japan) and Protect Bond (Kuraray) with Clearfil APX<sup>®</sup> (Kuraray) composite.

**Methodology** The direct contact test, with *E. faecalis*, *S. aureus*, and *P. aeruginosa*, was used. The walls of microtitre wells were coated with freshly mixed materials. The materials were tested immediately after application to the well (fresh samples) and after setting for 3 days (set samples). Ten  $\mu$ L bacterial suspensions were added to the wells for direct contact with the materials for 1 hour at 37°C. Growth of surviving bacteria was then measured in a microplate spectrophotometer hourly for 15 h at 620 nm. Autmixing before each reading ensured homogenous bacterial cell suspensions. Twelve uncoated wells using identical size of inoculum for the three bacteria served as positive control. Inhibition of bacterial growth was recorded either as a delay in outgrowth in the second plate and/or a lower final number of bacteria.

**Results** Fresh samples of all materials showed a 3-h delay in exponential growth of both *E. faecalis* and *S. aureus*, and a 5-hr delay in growth of *P. aeruginosa*. Set samples of IRM and ProRoot cements showed generally greater antibacterial activity than the other materials: both completely inhibited *P. aeruginosa*, and both delayed and limited growth of *E. faecalis*. IRM was active also against *S. aureus*, whereas ProRoot had little if any activity towards this organism.

**Conclusions** The direct contact test, by being quantitative and virtually independent of solubility and diffusion, was found more suitable to assay solid root-end filling materials. IRM and ProRoot MTA were generally more potent inhibitors of bacterial growth than the other materials.

## Research Posters – Microbiology

### R1

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#### Detection of bacteraemia associated with intra-canal and extra-canal file manipulation

**Aim** To determine the prevalence and intensity of bacteraemia associated with intraand extra-canal manipulation of instruments in asymptomatic teeth associated with periapical disease. The study was designed to account for confounding factors that may also potentially result in bacteraemia.

**Methodology** Blood and root canal samples were taken from 9 patients meeting strict selection criteria. The consented patients had a single asymptomatic tooth with apical periodontitis but were otherwise orally and generally healthy. Microbiological samples were taken from the root canals under aseptic conditions prior to root canal preparation using validated protocols. The first blood sample (baseline) was taken prior to any intra-canal filing. The root canals were then prepared to an apical size 20, maintaining the files within the terminus of the root canal system, determined by electronic and radiographic means. The second blood sample was taken 30 s following completion of this apical preparation. A size 15 instrument was then used as a patency file, 1 mm beyond the terminus of the root canal system. The third blood sample was taken 30 s after this. A period of 15 min was allowed to elapse between taking each blood sample. Blood samples were processed by lysis filtration and both blood and root canal samples were cultured under appropriate conditions. The prevalence and intensity of bacteraemia were determined.

**Results** Bacteria were isolated from all root canals sampled. Bacteraemias were not detected in the first (baseline) and second blood samples. Bacteraemias were detected in 5/9 (56%) of the third blood samples. The intensity of bacteraemia ranged from 1.0–5.0 CFU/6 ml.

**Conclusions** Only extra-canal manipulation of instruments caused bacteraemia, in just over half the cases, the intensity of which was low.

### R2

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#### Culture-dependent and culture-independent detection of persistent bacteria retrieved by a novel apical sampling method

**Aim** To compare culture-dependent and culture-independent techniques to detect persistent root canal bacteria following chemomechanical debridement, retrieved by a novel apical sampling protocol.

**Methodology** Nineteen single-rooted, previously untreated teeth with radiographic evidence of a periapical lesion were

subjected to root canal treatment and sampling procedures over 2 visits. Samples were obtained before and after separate preparation of the coronal (2/3) and apical (3 mm) parts of the canal using a protocol developed *in vitro*, which allowed independent sampling of the two parts of the canal. The coronal preparation was completed using ProTaper® and the apical using hand, stainless-steel instruments; NaOCl (2.5% w/w) irrigation and Ca(OH)<sub>2</sub> dressing were used in all cases. A further apical sample was taken just prior to root filling. Appropriate sterility-control measures were incorporated. Culture-dependent and culture-independent techniques were used to detect the presence of bacteria in the samples. The frequency of detection of bacteria in the apical samples using these techniques was compared using the McNemar test.

**Results** Apical preparation using 2.5% sodium hypochlorite and subsequent dressing with calcium hydroxide both resulted in a significant ( $P < 0.05$ ) reduction in the number of samples with cultivable bacteria. However, the culture-independent technique revealed no significant difference in bacterial presence (no differentiation between viable or dead) between samples taken before and after chemo-mechanical preparation. There was no significant difference in the detection of bacteria in the apical samples obtained after chemo-mechanical debridement using culture-dependent or culture-independent techniques.

**Conclusions** The study confirmed previous findings about the effect of chemo-mechanical debridement on the bacterial flora. There was no difference in the ability of culture-dependent and culture-independent techniques to detect bacteria in the apical samples after chemo-mechanical debridement.

### R3

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#### Microflora distribution in teeth associated with apical periodontitis determined by different microscopy techniques and protocols

**Aim** To compare two sample processing protocols to examine the presence and morphological distribution of bacteria in teeth associated with apical periodontitis, using three microscopy techniques [scanning electron microscopy (SEM), light microscopy (LM) and transmission electron microscopy (TEM)].

**Methodology** Nine root samples (7 teeth) were processed using either of two techniques (Eastman/Nair). The roots were sectioned longitudinally: the root portion designated for SEM was viewed along its entire length and descriptively divided into apical, middle and coronal thirds; semi-thin and ultra-thin sections were cut from the root portion designated for LM and TEM from each third of the root. Each third of the root was examined using all three microscopy techniques. Observations were systematically recorded.

**Results** Bacteria were detected in 8 of the 9 roots. A bacterial biofilm was a common finding on the root canal walls. Rods, cocci, filaments, fungal hyphae, spirochaetes and yeasts were observed. High magnification views revealed the nature of bacterial cell to cell contact via fimbriae and other cell membrane appendages. More bacteria were evident apically than coronally. Bacteria were seen in the dentinal tubules in the coronal and middle thirds but were not commonly found in the dentinal tubules apically. The roots processed using the Nair technique appeared to give more predictable results. Polymorphs (PMNs) were surprisingly found in all the root segments, mostly walling off the bacterial biofilm; although they were more commonly evident apically.

**Conclusions** The combination of three different microscopy techniques offered complementary views of the presence and morphological distribution of bacterial colonisation along the length of the root canal. The Nair protocol was superior to the Eastman technique in this study. The study provided further insight into the ecology of root canals and the nature and location of the host/microbial interface.

#### R4

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#### Species-richness of gram-positive coccoid morphotypes from root canals determined by phenotypic and genetic measures

**Aim** To compare the effect of microbial identification method on apparent species richness of gram-positive coccoid morphotypes recovered from untreated and treated root canals of teeth associated with periapical disease.

**Methodology** Gram-positive coccoid morphotypes ( $n = 177$ ) recovered from untreated ( $n = 105$ ) and treated ( $n = 72$ ) teeth from 12 patients were identified by physiological, biochemical and commercially available enzyme detection kits (Api, bioMérieux, Lyon, France) as well as by comparative 16S rRNA gene sequence (Partial) (Previously reported). The identities, their confidence values and biochemical test results (binary format) were analysed. The binary data were used to generate similarity matrices and dendrograms (PAUP) to compare the phenetic relatedness (TREEVIEW) of like-species from untreated and treated teeth.

**Results** The biochemical and molecular identities of 72% of strains matched at the genus level, 45% at the species level and 28% did not match. The method of identification influenced the species richness, regardless of the measure used (genus, species, confirmed or unconfirmed identification); 16S rRNA identification always increased the mean and range values per tooth. The greater species richness in this study compared to previous studies was probably due to a combination of sample type, method of sample retrieval, cultivation and identification procedures. A high 38% of isolates were given 'unacceptable' validity scores and were genus and sample-origin dependent. Bacteria from the treated teeth were more difficult to identify with a higher proportion of unacceptable scores (46% versus 32%),

suggesting absence of such profiles in the database. Lack of direct correlation between enzyme test profile and confidence score may suggest weighting of individual tests. The dendrograms showed that same-species strains from untreated and treated teeth were often phenotypically distinct.

**Conclusions** Commercial enzyme tests should not be used as the sole arbiter of their identity as frequently happens in endodontic microbiology; they should be supplemented with other confirmatory tests.

#### R5

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#### Antibiotic susceptibility of gram-positive coccoid morphotypes from untreated and treated teeth with periapical disease

**Aim** To determine the initial Minimum Inhibitory Concentrations (MIC) of commonly prescribed antibiotics for gram-positive coccoid morphotypes from root canals of untreated and treated teeth, using agar dilution and E-test; and to compare the MIC between and within test methods, bacterial groups and sample origins.

**Methodology** Gram-positive coccoid morphotypes from untreated ( $n = 107$ ) and treated ( $n = 77$ ) teeth (isolation procedures and identification previously reported) were subjected to antibiotic (Penicillin, amoxicillin, metronidazole, vancomycin, erythromycin, tetracycline, clindamycin and cefaclor) susceptibility testing using agar dilution (in accordance with the recommendations of the National Committee for Clinical Laboratory Standards) and E-test (AB Biodisk protocol, 1997). The MICs from the E-tests were plotted by antibiotic/strain combination to establish frequency distributions and their relationship to interpretive categories (NCCLS 2003). The MIC and resistance status were analysed by test method, bacterial group and sample origin.

**Results** There was broad agreement between the agar dilution and E-test methods (80% agreement within one doubling dilution, 90% agreement within two doubling dilutions). Frequency distribution plots showed the interpretive categories to be reliable. A total of 38% of the strains exhibited resistance to at least one of the 8 antibiotics tested and the majority (86%) of the resistant strains had multiple drug resistance. A higher proportion (53%) of strains from root-treated teeth was resistant compared to those from untreated teeth (26%); the main groups of resistant bacteria were: lactobacilli, staphylococci and enterococci. Antibiotic resistance profiles enabled strains from the same tooth to be differentiated into groups with possibly different origins. The resistant strains originated from many teeth, but a large proportion was from a select few teeth, possibly suggesting gene transfer between bacteria within root canals.

**Conclusions** E-test was comparable with an established method for determining MIC for root canal isolates. A high proportion of the strains exhibited resistance, especially in those from treated teeth.

## R6

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### Prevalence and transfer of tetracycline resistance gene [*tet(M)*] among root canal bacterial isolates

**Aim** To determine the prevalence of the tetracycline resistance gene [*tet(M)*] among tetracycline-resistant root canal isolates and to investigate the nature (conjugative transposon Tn916) and donor potential of Tn916-containing bacteria.

**Methodology** Tetracycline-resistant bacteria ( $n = 15$ ), previously obtained from teeth with apical periodontitis, were grown in pure culture and their total DNA extracted. Previously published primers were used to amplify the following genes [16S rRNA gene, *tet(M)*, Tn916 transposon-unique sites] by PCR using the DNA as template. The 16S rRNA gene provided the bacterial identification and successful amplification of the joint of circular Tn916 indicated its active status. Filter-mating experiments were carried out to assess the transfer of the Tn916 elements to recipient bacteria.

**Results** Of the original fifteen tetracycline-resistant bacteria selected, eight were shown by PCR to contain the *tet(M)* gene; four of these contained the unique Tn916 regions. Two of these elements were shown to be active by virtue of positive amplification of the 'joint' in a circular form. Filter-mating experiments successfully demonstrated the transfer of tetracycline resistance from one donor (*Neisseria* spp) to an enterococcal recipient.

**Conclusions** About half of the tested tetracycline-resistant bacteria had the *tet(M)* gene and about half of these demonstrated the presence of Tn916. Transfer of tetracycline resistance has been shown from one of these strains.

## R7

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### Induction of resistance by serial exposure of root canal isolates to sub-inhibitory doses of antiseptics

**Aim** To investigate induction of resistance and cross-resistance by serial exposure of root canal isolates to sub-inhibitory doses of antimicrobial agents.

**Methodology** Initial Minimum Inhibitory Concentrations (MIC) were calculated for 20 strains of bacteria isolated from untreated and treated teeth with apical periodontitis, using several antimicrobial agents (chlorhexidine gluconate, povidone-iodine, calcium hydroxide and sodium hypochlorite). Doubling dilutions of these agents were prepared in 96-well microtitre plates and the strains inoculated into the wells. After 72 hours, MIC values were recorded and strains from the 1/2 MIC well were sub-cultured on blood agar plates. The 1/2 MIC sub-cultures were re-inoculated into another 96-well plate and the exposure repeated; this was carried out 12 times. Following this serial exposure, the MICs were re-calculated and the strains maintained either by sub-culturing without

antimicrobial challenge or by freezing for one month. After 1 month the stability of the MICs was determined.

**Results** An increase in MIC was observed for 70% (14/20) of the strains for at least one antimicrobial agent following serial exposure; 35% (7/20) showed resistance to two (3/20) or three (4/20) antimicrobials. Considering the combination of strain and antimicrobial, 31% (25/80) showed an increase in MIC; of these 11 were resistant to chlorhexidine, 5 each to iodine and calcium hydroxide and 4 to sodium hypochlorite. The MIC changes were strain/antimicrobial-dependent but no obvious genus/species-dependent patterns were evident. The MIC for other antimicrobial agents also increased concurrently with that of the test agent, without exposure to them for 50% (10/20) strains. The induced change occurred for one specific or all of the non-test antimicrobials. The increase was stable after frozen storage but returned to pre-exposure levels on sub-culture without antimicrobial challenge.

**Conclusions** Serial exposure to sub-inhibitory doses of antimicrobials may cause reversible increases in MIC; withdrawal of exposure reduced the MIC to pre-exposure levels but not if the strains were frozen. Induced resistance may be accompanied by cross-resistance to other agents. The phenomenon was strain/antimicrobial-dependent without revealing definitive patterns.

## R8

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### Antimicrobial effect of calcium hydroxide, chlorhexidine digluconate and their combination in a human dentine block model

**Aim** To evaluate *in vitro* the antibacterial effect of calcium hydroxide (CH), chlorhexidine digluconate (CHX) and their combination (CH+CHX) against *Enterococcus faecalis* in a dentine block model with and without the use of EDTA.

**Methodology** Root canals of 48 human dentine blocks were prepared and smear layer removed by rinsing with EDTA (17%) and NaOCl (0.5%). The blocks were placed in Tryptic Soy Broth (Oxoid), autoclaved, inoculated with *E. faecalis* (A197A) and incubated at 37°C for 3 weeks. The blocks were rinsed with EDTA ( $n = 24$ ) or sterile water ( $n = 24$ ). In each group, 6 root canals were dressed either with CH (1 : 1 w/v in water), CH+CHX (1 : 1 w/v CH in 2% CHX), calcium carbonate (CC) +CHX (1 : 1.5 w/v) or CC (1 : 1.5 w/v in water) (negative control) for one week. Dentine samples obtained with a reamer were sampled in phosphate buffered saline. The samples were cultured on Tryptic Soy Agar (Oxoid) and the number of colony-forming units (CFU) was determined.

**Results** CH + CHX was the most effective dressing (mean  $\log_{10} \text{CFU} \pm \text{SD} = 2.11 \pm 2.06$ ), followed by CH ( $2.80 \pm 3.19$ ), CC + CHX ( $3.37 \pm 3.60$ ) and CC ( $4.66 \pm 4.36$ ). Rinsing with EDTA did not demonstrate antibacterial efficacy.

**Conclusions** CHX improves the efficacy of CH, whereas EDTA rinsing does not seem to have a direct killing potential in dentine disinfection. Quantification of bacteria residing in dentine is difficult as indicated by the high SD.

**R9**

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### Prevalence of oral enterococci among endodontic retreatment patients and dental students

**Aim** To investigate the prevalence of enterococci in four different sites of the oral cavity among patients undergoing root canal retreatment (P-group) and a group of dental students (S-group).

**Methodology** The P-group ( $n = 101$ , mean age 56 years) consisted of patients referred to the department of Endodontology for retreatment of apical periodontitis in a root filled tooth and showing >10 dental restorations. The S-group ( $n = 100$ , mean age 31 years), was recruited among dental students at Göteborg University with a high level of oral health (<4 dental restorations and no root filled tooth). Oral hygiene was classified as poor, fair or good and smoking habits were recorded. Microbiological samples were obtained from four different sites: subgingival and supragingival plaque, the tongue and cheeks. Samples were transported to the laboratory of oral microbiology using VMGA III and plated on Enterococcosel Agar. The specimens were incubated aerobically and bacterial growth was classified as present or absent.

**Results** Enterococci were more prevalent among individuals in the P-group (62%) than in the S-group (20%) ( $P < 0.001$ ). Enterococci were recovered from 144 sites and were most frequently identified in supragingival samples (54 sites) and in samples from the tongue (48 sites). In four individuals enterococci were present in samples from all sites and in 44 persons from only one site. In the S-group poor oral hygiene and smoking were factors associated with presence of enterococci ( $P = 0.002$  and  $P = 0.002$ , respectively). No such correlations were found in the P-group.

**Conclusions** Oral enterococci were common among patients undergoing root canal retreatment but were also found among a group of dental students with good oral health.

**R10**

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### Microbiological evaluation using T-RFLP analysis of the flora in necrotic teeth of patients irradiated in the head and neck region

**Aim** To evaluate the root canal flora in necrotic teeth in patients irradiated in the head and neck region by use of terminal restriction fragment-length polymorphism (T-RFLP) analysis and to compare the results with the flora from necrotic teeth in a control group.

**Methodology** Bacterial samples from necrotic root canals in patients treated for radiation caries following irradiation in the head and neck region (group a) and in healthy controls (group b) were analysed. Each sample in group a was taken from a different tooth (all were single rooted teeth, in total 13 root canals in 9 patients). Group b consisted of 11 patients of which 11 teeth were sampled (each time, 1 necrotic canal was sampled (in multi-rooted teeth, the root with easiest access was sampled). T-RFLP was used as identification technique. The 16S rRNA genes (rDNAs) of oral bacteria in root canals were amplified by PCR with universal primers. The 16S rDNAs were digested with the BstUI restriction enzyme and analysed by capillary electrophoresis (ABI310). T-RFLP patterns were numerically analysed using Basehopper, an in-house developed computer program. Bacterial species were assigned to terminal restriction fragments (T-RFs) by comparison with culture and cloning of 16S rRNA genes.

**Results** In group a, 3 samples were tested PCR negative, none in group b. A total of 50 different T-RFs were detected in the T-RFLP profiles (44 in group a and 28 in group b). Each T-RF represents one bacterial species or a cluster of bacterial species. Fifteen T-RF's could not be identified using the web-library. A mean of 13.20 T-RFs in group a and 6.55 T-RF's in group b were found per sample. This difference was statistically significant ( $P < 0.05$ ). A total of 22 different T-RF's were found in group a that were not found in group b. These mainly comprised of subspecies of *Lactobacillus* spp., *Capnocytophaga* spp. and *Actinomyces* spp.

**Conclusions** The flora in root canals of patients irradiated in the head and neck region (xerostomia) were significantly more diverse than the flora in a control group.

**Acknowledgements** This study was partly funded by the ESE Research Grant, Research Grant of the Fund for Scientific Research – Flanders (FWO – Vlaanderen) (n°1.5.126.04) and the Vlaamse Vereniging voor Tandartsen.

**R11**

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### Assessment of the bacterial load in root canals using real-time PCR

**Aim** To evaluate the bacterial load of root canals with the diagnosis of chronic apical periodontitis by means of quantitative real-time PCR depending on the method for root canal disinfection.

**Methodology** Twenty teeth with radiographically documented periapical lesions were included. Ten of these teeth had a deficient root filling; the other 10 had not been treated. After removal of either pulp tissue or root canal filling, bacterial samples were obtained with sterile paper points, using measures to prevent contamination. Root canals were prepared using rotary instruments. During preparation, in each group 5 teeth were rinsed with either NaOCl 2% or chlorhexidine 0.1%. A second set of samples were taken immediately after treatment and the third samples after a period of 14 days during which the canals were filled with a calcium hydroxide dressing.

**Results** In the NaOCl group the mean total number of bacteria was  $1.96 \times 10^7$  (SD:  $5.64 \times 10^7$ ) before root canal preparation. The bacterial load was statistically significant reduced to  $4.56 \times 10^4$  (SD:  $9.33 \times 10^4$ ) immediately after treatment ( $P < 0.05$ ). After the 14 days period during which the root canals were filled with calcium hydroxide, the number of bacteria was  $3.9 \times 10^5$  (SD:  $8.29 \times 10^5$ ). In the chlorhexidine group the corresponding values were  $4.86 \times 10^7$  (SD:  $6.33 \times 10^7$ ) before root canal preparation,  $2.66 \times 10^4$  (SD:  $2.42 \times 10^4$ ) immediately after treatment and  $8.77 \times 10^3$  (SD:  $6.85 \times 10^3$ ) after 14 days ( $P < 0.05$ ). In teeth with insufficient root canal fillings, the total number of bacteria was lower and the reduction of the bacterial load was not statistically significant.

**Conclusions** The novel method of quantitative real-time PCR appears to be well suited to evaluate various disinfection protocols during root canal treatment.

## R12

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### The effect of canal filling with Gutta-percha or Resilon on *Enterococcus faecalis* in bovine dentinal tubules

**Aim** To observe the effect of canal filling on survival of *E. faecalis* remaining in dentinal tubules and to compare the sealing ability of Gutta-percha and Resilon (SybronEndo, USA).

**Methodology** Bovine teeth were sectioned to produce specimens 4 mm thick with 6 mm external diameter and 2.3 mm of internal diameter. *E. faecalis* was inoculated into the specimens and incubated. The outer surface was coated with nail varnish and the internal diameter was increased to 2.5 mm by using an ISO 025 round bur. The dentine specimens were divided into 5 groups by canal filling method. Group 1 was the negative control. Group 2 was the positive control. In Group 1 and 2, the internal surface of the dentine blocks was coated with nail varnish. Group 3 was filled with ZOE based sealer and Gutta-percha, Group 4 with resin based sealer and Gutta-percha, and Group 5 with resin based sealer and Resilon. After 24 h at room temperature, the blocks were incubated at 37°C for 1, 2, 3 and 4 weeks on BHI agar plates. The internal layer of the dentine blocks was removed using ISO 027, 029, 031, 035 round burs and the resultant dentine chips were incubated on culture medium at 37°C for 24 h. Following incubation, the optical density of the medium was measured using spectrophotometer.

**Results** There was a statistically significant reduction in the number of *E. faecalis* in the group where dentinal tubules were completely sealed with nail varnish in comparison with the groups obturated with gutta-percha or Resilon. In group 5, the number of *E. faecalis* in the dentinal tubules decreased significantly with time, whereas in group 3 and 4, there was no reduction.

**Conclusions** Canal sealing ability of both Gutta-percha and Resilon was not complete since *E. faecalis* in dentinal tubules survived after canal filling. Evidence emerged that the resin based sealer and Resilon would exhibit a better root canal sealing ability in the long-term.

## Research Posters – Canal Preparation

## R13

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### Procedural errors during shaping root canals to increased apical preparation sizes using Lightspeed rotary instruments

**Aim** To evaluate the incidence of procedural errors following root canal treatment with Lightspeed (LS) rotary instruments with increased apical enlargement.

**Methodology** A total of 80 patients (117 teeth with 287 roots) underwent root canal treatment which was carried out under standardized conditions by a trained operator using Lightspeed rotary instruments (85 molars, 25 premolars, 7 incisors). The apical portion was enlarged to sizes varying on average between sizes 40 to 60. The mean apical preparation size was 52.5. Sixty-three teeth were recalled after a mean interval of 25 months. Initial and recall radiographs were

assessed using the periapical index (PAI). Procedural errors such as instrument fractures, perforations and root canal transportation were noted.

**Results** Only three LS instrument fractures occurred (sizes 37.5, 40, 52.5), all in the apical portion of the canal. The presence of the fractured LS instruments had no impact on apical healing. One perforation was noted in the middle third of the canal. No root canal transportation was observed.

**Conclusions** In the hands of a trained operator, root canal treatment with Lightspeed rotary instruments was a safe technique, allowing apical enlargement to sizes larger than commonly recommended.

**R14**

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### Shaping ability of ProTaper in comparison to a ProTaper–Profile combined technique in simulated root canals

**Aim** To compare the shaping ability of ProTaper (Dentsply Maillefer, Switzerland) instruments and the combined use of ProTaper and ProFile (Dentsply Maillefer) instruments in severely curved simulated root canals.

**Methodology** Thirty simulated canals with curvature of 40° and different shapes in terms of position of curvature (straight section before curve: 8 and 12 mm) were prepared using two different preparation methods: group A with the ProTaper instrument set (apical preparation to F3) according to the recommendations of the manufacturer; group B with ProTaper preparation of the coronal two thirds (S1 to F1) and apical enlargement with ProFile .04 taper (sizes 20–35). Pre-operative, sequential and post-operative pictures, recorded using a digital camera, were superimposed. Measurements were carried out at 5 different points: canal orifice (O); half-way to the orifice in the straight section (HO); beginning of the curve (BC); apex of the curve (AC); endpoint (EP).

**Results** In both canal types the total canal width was significantly higher in group A compared to group B at points HO, BC and AC ( $P < 0.05$ ); a similar result was found for the transportation of the central axis at the apex of the curve (AC,  $P < 0.05$ ). Mean transportation was towards the inner aspect of the curve in all canal types at BC, towards the outer aspect in canals with 12 mm straight section at AC. In both canal types, the mean transportation was significantly higher in group A at point AC ( $P < 0.05$ ).

**Conclusions** Under the conditions of this study, apical enlargement of curved canals with ProFile .04 taper instead of ProTaper instruments resulted in less apical transportation and a more centred preparation shape in and beyond the apex of the curve.

**R15**

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### *In vitro* comparison of four different instruments for preparation of simulated curved root canals

**Aim** To compare the shaping ability of Hero 642, Hero Shaper (Micro-Mega, France), NiTi-TEE (Tapered file System, Sjöding Sendoline AB, Sweden) rotary nickel titanium instruments and stainless steel Hand K-Files (Dentsply Maillefer, Switzerland).

**Methodology** A total of 60 simulated canals with 42° curves (Endo training block; Dentsply Maillefer Instruments) were prepared with either Hero 642, Hero Shaper and NiTi-TEE rotary nickel titanium instruments using a crowdown preparation technique according to the manufacturers instructions or with K-files using a stepback technique ( $n = 15$  for

each group). All canals were prepared up to an apical size 30. Preparation time, changes of working length and instrument failures were recorded. In addition, post-instrument images were taken and assessment of the canal shapes was examined by a computer image analysis program (UTHSCSA Image Tool for Windows, University of Texas, USA).

**Results** Four NiTi-TEE instruments and one K-file separated; none of the Hero 642 and Hero Shaper instruments separated. All Ni-Ti instruments were significantly faster and maintained working distance better than K-files. In comparison with stainless steel K-files all Ni-Ti rotary instruments maintained the taper of root canals.

**Conclusions** Hero 642, Hero Shaper and NiTi-TEE instruments prepared curved root canals rapidly, without changing working length and maintained the taper of root canal. Some NiTi-TEE instruments fractured.

**R16**

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*ACTA, Amsterdam, The Netherlands*

### The efficacy of ultrasonic irrigation to remove artificially placed dentine debris from human root canals prepared using GT rotary instruments with different tapers

**Aim** To investigate the influence of diameter and taper of root canals on the effectiveness of ultrasonic irrigation to remove artificially placed dentine debris from different-sized root canals.

**Methodology** Forty-four maxillary and mandibular canines were selected after bucco-lingual and mesio-distal radiographs indicated that their internal diameters were smaller than the diameters of a size 20, .06 taper GT instrument. These canines were divided into three groups and prepared using either size 20, .06 taper GT rotary instruments, size 20, .08 or size 20, .10 GT instruments. Each root was then split longitudinally through the canal, forming two halves. In one canal wall, a standard groove was cut 2 to 6 mm from the apex, to simulate uninstrumented canal extensions. Each groove was filled with dentine debris mixed with 2% NaOCl to simulate a situation when dentine debris accumulates in the uninstrumented canal extensions. Each canal was reassembled by joining the two halves of the teeth by means of wires and sticky wax. In each canal ultrasonic irrigation was performed using 2% NaOCl as irrigant. Before and after irrigation, images of each half of the canal with a groove were taken using a microscope and a digital camera, after which they were scanned into a PC as TIFF images. The quantity of dentine debris in the groove was evaluated using a scoring system: the higher the score, the larger the amount of debris. The scores before and after irrigation were compared.

**Results** After ultrasonic irrigation, the debris score reduced by 73.9%, 80.9% and 92.7% respectively in the size 20, .06, 20, .08 and 20, .10 taper groups. However, the difference amongst groups was not statistically significant ( $P = 0.078$ ).

**Conclusions** There was a tendency for ultrasonic irrigation to be more effective in removing artificial dentine debris from canals with greater taper.



## R17

H. G. Kuah<sup>\*1</sup>, S. K. Tseng<sup>2</sup> & N. N. Chen<sup>1</sup><sup>1</sup>National Dental Centre & <sup>2</sup>National University of Singapore, Singapore**Effect of chelating agents on smear layer removal with and without ultrasonics****Aim** To evaluate *in vitro* the effectiveness of chelating agents on smear layer removal with and without the use of ultrasonics.**Methodology** One hundred and five extracted teeth were randomly divided into 7 groups. They were mechanically instrumented to apical file size 40 with a combination of rotary and hand instrumentation. RC-Prep and 1% sodium hypochlorite (NaOCl) were used during instrumentation in all groups except for Group 1, where only saline was used. All 7 groups had different final irrigating protocols, which were as follows: Group 1: Physiological solution (saline) for 3 min with ultrasonics followed by saline; Group 2: NaOCl for 3 min followed by NaOCl; Group 3: NaOCl for 3 min with ultrasonics followed by NaOCl; Group 4: EDTA for 3 min followed by NaOCl; Group 5: EDTA for 3 min with ultrasonics followed by NaOCl; Group 6: EDTA for 1 min followed by NaOCl; Group 7: EDTA for 1 min with ultrasonics followed by NaOCl. The specimens were then vertically grooved, split and prepared for SEM examination. Specimens were evaluated at the apical (2 mm) and mid-root (6 mm) levels at 1000× and 3000× magnification. Smear layer and debris removal were scored by 2 examiners based on the patency of dentinal tubules and amount of debris observed. Results were subjected to statistical analysis using Chi-square / Fischer's Exact Test.**Results** At the 2 mm level, only specimens from groups with EDTA and ultrasonic irrigation (Group 5 and 7) had significantly more specimens with smear layer and complete debris removal compared to the other groups. At the 6 mm level, regardless of the use of ultrasonics, the groups with EDTA irrigation (Group 4, 5, 6 and 7) had more specimens with smear layer removed. However, only specimens with EDTA and ultrasonic irrigation (Groups 5 and 7) had a significantly higher number of specimens with complete debris removal at the 6 mm level.**Conclusions** Within the limitations of the study, the combined use of EDTA and ultrasonics is more efficacious for smear layer and debris removal especially in the apical region. A one minute application of ultrasonics in the final irrigation sequence appeared to be sufficient for this purpose.**Acknowledgements** A/P V Sac-Lim, Dr Chan YH, Prof. G Bergholtz.

## R18

J. Tanalp<sup>\*1</sup>, F. Kaptan<sup>1</sup>, B. Kayahan<sup>1</sup>, S. Sert<sup>2</sup>, M. Karapinar Kazandag<sup>1</sup> & G. Bayirli<sup>1</sup><sup>1</sup>Department of Endodontics, Faculty of Dentistry, Yeditepe University, Goztepe & <sup>2</sup>Gulhane Military Academy, Dental Service, Uskudar, Istanbul, Turkey**Scanning Electron Microscope evaluation of the effectiveness of Glyde File Prep<sup>®</sup> on the removal of the smear layer****Aim** To evaluate the effectiveness of 'Glyde File Prep<sup>®</sup>' in the removal of smear layer produced by rotary instruments.**Methodology** Thirty extracted human mandibular canine teeth were used. The teeth were divided into three groups of 10 teeth. Instrumentation was performed with Protaper (Dentsply Maillefer, Switzerland) rotary instruments. The final instrument used at the working length was size 30. Irrigation was performed as follows: group 1: Routine irrigation with 2 mL of 2.5% NaOCl and 2 mL of 17% EDTA after each file + Final irrigation with 10 mL of 17% EDTA and 2.5% NaOCl + Distilled water. Group 2: Each file coated with 'Glyde - File Prep<sup>®</sup>' before instrumentation + Final irrigation with 10 mL of 17% EDTA and 2.5% NaOCl + Distilled water. Group 3 (Control): Routine irrigation with 2 mL of 2.5% NaOCl after each file + Final irrigation with 10 mL of 2.5% NaOCl Distilled water. The teeth were split longitudinally into 2 halves. The coronal, middle and apical root surfaces were evaluated under scanning electron microscope at ×500 and ×1500 magnifications and scored for smear layer using a point scale. The efficacy of the irrigant regime in terms of smear layer removal was evaluated.**Results** Statistical analysis revealed a significant difference between the coronal, middle and apical aspects within each group at both magnifications ( $P < 0.05$ ). Less smear layer removal was observed in the apical level in both experimental groups. No statistically significant difference was observed in terms of smear layer removal between the experimental groups.**Conclusions** No significant difference existed in terms of smear layer removal when Glyde was compared with the traditional NaOCl + EDTA regime.**Acknowledgements** The authors would like to thank Tubitak Gebze Research Institute for their contribution in this study.

## R19

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**Efficiency of a novel rinsing device for the removal of pulp tissue *in vitro*****Aim** To assess the efficiency of a novel pneumatic device for root canal rinsing by evaluating the amount of remaining adherent pulpal tissue on the canal surfaces.**Methodology** The roots of 90 extracted teeth were divided into two groups and prepared up to size 20 or 40 using hand instruments. Teeth were not rinsed during root canal preparation procedures. After completing the preparations, root canals were randomly assigned to 3 groups: pneumatic

rinsing with NaOCl 2% for 1 min (Endorinse, Duerr, Germany), conventional rinsing by hand using a syringe with NaOCl 2% for 1 min, no rinsing (control). Teeth were fixed in formalin and embedded in a light-activated PMMA medium. Sections of 30  $\mu$ m were cut at intervals of 2, 4 and 6 mm from the apex. After staining the sections with toluidine blue the specimens were examined by light microscopic analysis. For each root canal the amount of unprepared root surface with and without adherent pulpal tissue was evaluated. Additionally, the amount of prepared root surface with and without a smear layer was determined.

**Results** Regarding the non-prepared surfaces, the amounts of residual pulpal tissue in the control group (size 20:  $93 \pm 10\%$ , size 40:  $95 \pm 10\%$ ) and after conventional rinsing (size 20:  $91 \pm 14\%$ , size 40:  $95 \pm 10\%$ ) were not statistically different ( $P > 0.05$ ). Employing the pneumatic device (size 20:  $75 \pm 26\%$ , size 40:  $76 \pm 16\%$ ), statistically significantly less pulpal tissue was found in the size 40 group ( $P < 0.05$ ). Evaluating the prepared surfaces, the smear layer was removed similarly in all groups rinsed with NaOCl.

**Conclusions** The present study indicates that the novel pneumatic device is capable of removing pulpal tissue more efficiently than conventional rinsing by hand by reducing the amount of adherent pulpal tissue. The system could improve disinfection procedures during root canal treatment.

## R20

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### An *in vitro* investigation of the accuracy of two new apex locators

**Aim** To investigate the accuracy of two new electronic apex locators (EAL), Elements (SybronEndo, USA) and Propex (Dentsply Maillefer, Switzerland), and to compare these results with the radiographic technique and a well-known apex locator, Root ZX (J Morita, Japan) using an *in vitro* model.

**Methodology** Thirty anterior teeth with straight canals stored in a 10% formalin were used. The coronal part of each tooth was reduced to create a fixed reference point. Access cavities were prepared and the actual lengths (ALs) were measured. The teeth were then embedded in an alginate model. Electronic tooth length measurements (ELs) were carried out using the three EALs. The measurements were repeated 3 times and an average computed. Periapical radiographs of each tooth were taken using a digital sensor and the radiographic lengths (RLs) were measured 0.5 mm short of the radiographic terminus. The teeth were then divided into 3 groups of 10 teeth each. All canals were enlarged to a size 40 K-file for 2/3rd of the canal length. Saline was used as irrigant. In group 1, EL measurements were then taken three times by each EAL in dry condition, in group 2 and 3, with saline and sodium hypochlorite in the canals respectively.

**Results** The results showed that all the EALs were highly accurate to within +0.5 mm of the apical foramen, with mean differences between the AL and EL by Root ZX was 0.31 mm

(SE = 0.05), Elements 0.23 mm (SE = 0.04) and Propex 0.36 mm (SE = 0.07). RLs were significantly less accurate compared to the readings from all the EALs. No significant difference was found in the reading between the three apex locators when measurements were taken in dry canals and the sodium hypochlorite solution did not affect the accuracy of the measurements.

**Conclusions** Both Elements and Propex proved to be as reliable as Root ZX and were user friendly.

## R21

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### Accuracy of three apex locators in root-end resected teeth

**Aim** To assess the accuracy of apex locators in determining the working length in root-end resected teeth.

**Methodology** Ninety extracted human posterior teeth with 182 root canals were used. The root canal system was accessed, the canals prepared to a minimum of size 40 and then filled with gutta-percha and sealer. The apical 3 mm of root was then resected and the root fillings removed using Hero NiTi rotary instruments (MicroMega, France). The size of the apical terminus of the root canals following removal of the root fillings was between 50 and 90. The apical terminus of the root canal was then determined using 3 apex locators (Root ZX, J Morita; Raypex<sup>®</sup>4, VDW Germany; and Apex Pointer, Micro Mega, France). The media used for the measurements was isotonic salt solution (0.9% NaCl). A new mounting model that utilizes a micrometer was used to perform the measurements. The repeatability of each apex locator and the inter-operator agreement was determined.

**Results** All apex locators had a clinically acceptable repeatability; (0.03 mm coefficient of repeatability) and narrow limits of interoperator agreement (+0.07 and -0.07 mm). The accuracy of determining the apical terminus within 1 mm in the root canal was as follows: Root ZX 90% (164/182 root canals) [95% CI: 86%; 94%], Raypex<sup>®</sup>4 74% (135/182) [95% CI: 68%; 80%], and Apex Pointer 71% (129/182) [95% CI: 65%; 77%]. No overinstrumentation resulted when the Root ZX device was used. In contrast, using the Raypex<sup>®</sup>4 or the Apex Pointer device resulted in overinstrumentation in 8 out of 182 root canals (4%).

**Conclusions** All three apex locators were able to detect the apical terminus of root-end resected teeth within a clinically acceptable range, however; the Root ZX device had the greatest accuracy and resulted in no over-instrumentation of the root canal.

## R22

J. Jan\*<sup>1</sup> & D. Krizaj<sup>2</sup><sup>1</sup>Department of Endodontics, Medical Faculty & <sup>2</sup>Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia**Influence of canal wall thickness on electronic working length determination****Aim** To investigate the influence of canal wall thickness on working length determination with the impedance method.**Methodology** Electrical impedance was measured *in vitro* on three extracted single-canaled human teeth using an HP 4284 LCR meter (Hewlett Packard, USA). A file electrode was inserted into the root canal, the second electrode was placed in the saline solution surrounding the tooth. Measurements were performed at frequencies from 500 Hz to 20 kHz and repeated with different distances of the file from the apical foramen. From the data, electrical lumped element models were developed. Numerical simulation with Femlab (Comsol, Sweden) was performed to investigate the influence of the canal wall thickness on current conduction through the tooth.**Results** With smaller thickness of the dentinal canal wall electrical conductance was increased and a reduction in impedance due to current flow directly through the dentine wall was observed. Changes in impedance caused by varying the distance of the file from the apical foramen were smaller and more difficult to interpret, which reduced the accuracy of the method. The use of impedance ratios measured at different frequencies improved the accuracy of apical foramen determination.**Conclusions** Thickness of the canal wall is an important parameter influencing the accuracy of the impedance method for working length determination in root canal treatment.**Acknowledgements** Supported by the Slovenian Ministry of Science, Education and Sport (No. J3-8713-0381-99).

## R23

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**Apical extent of rotary canal instrumentation with an apex locating engine-driven canal preparation system****Aim** To evaluate the apical extent of rotary canal instrumentation using the Dentaport (J Morita, Japan) at different automated settings.**Methodology** Forty extracted human teeth with single roots with apparently mature apices were selected. Teeth were embedded into freshly mixed alginate mould. The canal lengths were measured initially using the Dentaport apex locator to the apical constriction and then instrumented with nickel titanium rotary files. For the instrumentation the automatic apical reverse mode was set to 0.5 (group A) and 1 (group B) that corresponded to different distances from the apical foramen (20 teeth in each group). Then a file was secured at the working length with a composite material. Thetip of a size 10 K-file was introduced to the canal 2 mm deep from the apex side to mark the localization of the apical foramen. The apical 4 mm of the canal of each tooth was exposed by grinding with a water-cooled, high-speed diamond bur. Distances from the file tip to the apical foramen were measured with the use of an endodontic microscope (Karr, Switzerland) under 17-fold magnification. Paired *t*-tests were used to compare the results from both experimental groups.**Results** When the automatic apical reverse mechanism's setting was 0.5, the instrumented length was 0.31 shorter than visually measured lengths and 0.2 shorter than electronically measured length. When the automatic apical reverse mechanism's setting was 1, the instrumented length was 0.43 mm shorter than visually measured lengths and 0.3 mm shorter than electronically measured length. There was a statistically significant difference between both groups ( $P < 0.05$ ). Eight canals were instrumented up to apical foramen in group A and only in one in group B.**Conclusions** The apical extent of rotary canal instrumentation was more consistent when the automatic reverse setting mechanism was set to 1.

## R24

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**The effect of apical root-end preparation on crack formation****Aim** To evaluate the effect of ultrasonic and bur apical root-end preparation on dentine crack formation.**Methodology** Eighty single rooted teeth were prepared chemomechanically and filled with gutta-percha and AH Plus (Dentsply DeTrey, Germany) sealer using a lateral condensation technique. The root-ends were resected with a fissure bur. All teeth were randomly divided in two equal groups. In group 1 root-end cavities were prepared to a depth of 3.0 mm using a 010 inverted cone steel bur mounted on a slow speed micro-handpiece. In group 2 root-end cavities were prepared with S12/90.D tips (Satelec, France) at the intensity recommended by the manufacturer (Power 7 at power mode S). All resected surfaces were analyzed and photographed using an endodontic microscope OPM 1F (Karr Dental, Switzerland) at  $\times 34$  and  $\times 60$  magnification before and after root-end preparation. Differences in the numbers of cracks following root-end preparation compared to those seen after root resection were recorded and analysed using the Fisher exact test ( $P < 0.05$ ).**Results** Dentine cracks before root-end preparation were noted in 14 roots of group 1 and in 11 roots of group 2. An increase in the number of cracks was present only in teeth prepared with ultrasonic tips (group 2) with new cracks developing in 13 teeth. Bur root-end preparation revealed no crack formation.**Conclusions** Ultrasonic root-end preparation increased the incidence of dentine crack formation compared to bur root-end preparation.

## Research Posters – Filling

### R25

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#### The influence of irrigant solution volume used during passive ultrasonic irrigation on the quality of root fillings

**Aim** To investigate the quality of root fillings following passive ultrasonic irrigation using three different volumes of irrigant solution.

**Methodology** Three groups of 20 oval shaped distal root canals of mandibular molars were cleaned and shaped and passive ultrasonic irrigation performed using three different volumes of sodium hypochlorite (NaOCl) namely 50, 120 and 200 mL. The root canals were dried with paper points and filled with AH26 and gutta-percha using a warm vertical compaction technique. The roots were sectioned horizontally 2, 4 and 6 mm from the apex of the tooth using a low-speed saw. The sections were photographed using a digital camera and pictures of the sections were taken under a Photomicroscope M400 microscope at  $\times 40$  magnification. These photographs were then scanned as Tagged Image File Format (TIFF) images. Using a KS 100 Imaging system 3.0 the area of the canal and gutta-percha were outlined by hand and then measured. The percentage of gutta-percha filled area (PGP) was calculated. The data was analysed using multiple linear regression models; the PGP was the dependent factor; the volume of irrigant and the level of section were covariates.

**Results** The volume of irrigant did not influence the values of PGP ( $P = 0.978$ ), whereas the sectioning level did ( $P = 0.000$ ). The average PGP was 87.4%, 95.6% and 95.1% at 2, 4, and 6 mm respectively.

**Conclusions** There is no significant difference in the quality of the root filling after using volumes of 50, 120 or 200 mL of NaOCl solution during passive ultrasonic irrigation.

### R26

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#### A 1-year follow-up study on leakage of single-cone fillings with RSA RoekoSeal

**Aim** To measure long-term leakage along single cone fillings.

**Methodology** Two groups of canines ( $n = 30$ ) were prepared to a size 55 master apical file. Stepback was performed using files of sizes 60, 70 and 80. The canals were filled using a single cone technique using RoekoRSA (Coltene Whaledent Roeko, Germany) as sealer. A bi-directional spiral was used in one group to place sealer into each canal whereas a gutta-percha cone was used in the other group. Immediately after root filling, the coronal portion of root filling was removed during post-space preparation. After one week and one year, leakage along 4 mm of the remaining apical root filling was

measured using a fluid transport model. Ten additional canine roots were prepared and filled with gutta-percha cones without sealer, serving as positive controls. Occurrence of apical extrusion of materials was recorded.

**Results** The apical root filling in all 60 canine roots did not show leakage either at one week or at one year. All ten positive controls showed gross leakage ( $>20 \mu\text{L/h}$ ). In no cases was gutta-percha extruded through the apical foramen. Sealer extruded apically in 88% of the roots where a gutta-percha cone was used to introduce sealer and in 28% of the roots where a bi-directional spiral was used to introduce sealer (chi-square test,  $P < 0.01$ ).

**Conclusions** In wide and straight canals single cone fillings with RoekoRSA sealer prevented fluid transport for one year. Using a bi-directional spiral to place sealer reduced sealer extrusion.

### R27

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#### A meta-analysis of the sealing ability of various root canal sealers

**Aim** To compare the sealing ability of various root canal sealers based on reliable *in vitro* research papers and to reveal possible parameters that may influence their results.

**Methodology** The search of the articles was performed via MEDLINE for the years 1980–2003. Key words were 'root canal sealers', 'endodontic microleakage', 'endodontic leakage', 'root canal sealers and microleakage', 'root canal sealers and leakage', 'obturation and microleakage', 'obturation and leakage'. All full articles were then thoroughly evaluated, in order to assess whether they fulfilled the pre-requisites of the study. Statistical analysis determined the quantitative differences of leakage among various sealers in the basis of fixed effects or random effects, depending on the homogeneity of each individual study. When possible, metaregression models were applied. Data analyzed included mean value and standard deviation of leakage of each specimen tested, number of specimens, sealer used, removal of smear layer or not, obturation technique and method of leakage measurement, including details on the dye and direction of measurement.

**Results** Of the initial 93 articles, 46 fulfilled all the inclusion criteria. Statistical analysis showed that zinc oxide-eugenol sealers possess inferior sealing ability compared to epoxy resin-based and calcium hydroxide-based sealers, whereas no significant differences were revealed between zinc oxide-eugenol and glass ionomer based sealers. A common finding in the paired comparison was that the results were not influenced either by the filling technique or from the direction of leakage. It also showed that dye leakage techniques were not reproducible, while type of the dye and removal of smear layer influenced the values of leakage.

**Conclusions** The findings imply that the critical role of sealers in the achievement of a seal is fully justified.

## R28

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### Sealing ability of different gutta-percha techniques: Thermafil, Quick-Fill, Soft-Core, Microseal, SystemB and lateral condensation technique

**Aim** To compare apical microleakage of roots filled with 6 different gutta-percha techniques; Thermafil (Dentsply Maillefer, Switzerland), Quick-Fill (JS Dental Manufacturing Inc, USA), Soft-Core (Soft Core System, Denmark), Microseal (Tycom, USA), SystemB (Sybron Endo, USA) and lateral condensation, using a fluid filtration system.

**Methodology** Sixty single rooted teeth were used for this purpose. Six groups of 10 teeth were randomly filled by either Thermafil, Quick-Fill, Soft-Core, Microseal, System B or lateral condensation techniques. After 2 years of storage in 100% humidity, a fluid filtration system was used to evaluate apical leakage. Filtration rate was measured by the movement of an air bubble in a micropipette for 5 min. intervals under a pressure of 120 kPa (1.2 atm). Measuring was performed four times for each specimen. The mean values were determined and expressed as  $\mu\text{cmH}_2\text{O}^{-1} \text{ min}^{-1}$ . All data were fed into PC-compatible software and analyzed statistically using ANOVA followed by Duncan tests ( $P < 0.05$ ). The negative control group was tested with a root of which the apex was covered to ensure that there was no leak in the device.

**Results** Thermafil, Soft-Core, Quick-fill, SystemB techniques were superior to Microseal and lateral condensation techniques ( $P < 0.05$ ). Although Microseal had the most microleakage, no statistically difference was found between Microseal and lateral condensation ( $P > 0.05$ ). Thermafil had the least leakage, but no statistically difference was found between Thermafil, Quick-Fill, Soft-Core and SystemB techniques ( $P > 0.05$ ).

**Conclusions** In the present study, Thermafil, Quick-Fill, Soft-Core and System B techniques had significantly less apical leakage than Microseal and lateral condensation techniques.

## R29

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### Comparative study of sealing ability of a new resin-based root canal sealer

**Aim** To compare the sealing ability of three root canal sealers: Epiphany (Jeneric/Pentron, USA), Tubliseal (Kerr, USA) and Sealapex (Kerr).

**Methodology** Sixty single-rooted human teeth were used. The root canals were prepared with the stepback technique. The working length was the same for all specimens and a size 35 was used to prepare the apical end-point. NaOCl (5%) was used as the irrigant solution after use of each instrument. All specimens were stepped back to a size 60. A final irrigation with 17% EDTA (3 mL/3 min) was used to remove smear layer. The teeth were divided into three groups. Teeth in group A were filled with laterally condensed gutta-percha using Epiphany (resin sealer), teeth in group B were filled with gutta-percha and Tubliseal (zinc-oxide eugenol sealer) and teeth in group C were filled with gutta-percha and Sealapex (calcium hydroxide sealer). Six additional specimens were used as controls. All the test sealers were mixed and used according to the manufacturers' instructions; the canals were filled by one operator. Microleakage was measured using a fluid transport system after 7 days and 1 month. Between measurements the specimens were stored in distilled water at 37°C.

**Results** At 7 days the group filled with the Epiphany system leaked less than those filled with Tubliseal and Sealapex ( $P < 0.05$ ); no statistically significant difference was found between the Tubliseal and Sealapex groups. At 1 month the group filled with the Epiphany system leaked less than those filled with Tubliseal and Sealapex ( $P < 0.05$ ).

**Conclusions** Epiphany sealer allowed significantly less leakage than Tubliseal and Sealapex.

## R30

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### The long-term sealing ability of two root canal sealers using different filling techniques

**Aim** To evaluate the long-term sealing ability of AH Plus (DeTrey, Germany) and RoekoSeal Automix (RSA) (Roeko Dental Products, Germany) when used with different filling techniques.

**Methodology** The experiment was conducted on sixty-six extracted single rooted teeth. The crowns were removed at the cemento-enamel junction and root canals were instrumented using a stepback technique. Samples were divided into three groups of 20 teeth each and root canals were filled using either cold lateral condensation, the Touch'n Heat technique or the Thermafil technique. For each technique AH Plus was used as the sealer in 10 samples and in 10 samples RSA. Six sample were used as the control group. With the use of a fluid transport model leakage was measured repeatedly at 1 month, 6 and 12 months and recorded in microlitres per day.

**Results** Leakage was significantly greater ( $P < 0.05$ ) after month 12 compared to month 1, for all techniques and with both sealers. There was no significant difference between sealing ability of AH Plus and RSA.

**Conclusions** RSA and AH Plus in combination with either cold laterally compacted gutta-percha, Touch'n Heat and Thermafil provided similar sealing ability. Both materials leaked more after 12 months.

### R31

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### Leakage analysis of three modern root filling materials after 90 days of storage

**Aim** To evaluate the apical seal of root fillings with GuttaFlow, Epiphany and RelyX Unicem after a storage time of 90 days.

**Methodology** Thirty extracted human single-rooted teeth were prepared up to size 45, .04 taper with FlexMaster (VDW, Germany) and ProFile (Dentsply Maillefer, Switzerland) instruments. Teeth were randomly assigned to three groups ( $n = 10$ ): group 1: GuttaFlow (Coltène/Whaledent, Langenau, Germany), group 2: Epiphany (Pentron, Wallingford, CT, USA), group 3: RelyX Unicem (Maxicap) (3 M ESPE, Seefeld, Germany). Final irrigation sequences were performed as follows: group 1: 40% citric acid, 5% NaOCl, 70% ethanol; group 2: 5% NaOCl, 17% EDTA, sterile water; group 3: 40% citric acid, 5% NaOCl, sterile water (2 mL each). Canals were dried with absorbent paper points. Root canal filling materials were placed with a lentulo spiral. A single cone (size 40, .04) of gutta-percha or Epiphany (group 2) was inserted. Teeth were stored in 100% humidity at 37°C for 90 days. Microleakage was evaluated using a dye penetration test (methylene blue 5% / centrifuging at 30 g for 3 min). After cross sectioning in steps of 1 mm specimens were evaluated for linear dye penetration under a stereomicroscope (40×). The maximum leakage value possible was 9 mm.

**Results** Linear dye penetration (mm) resulted as follows (mean/ SD): group 1 (2.2/0.42); group 2 (5.5/3.1); group 3 (3/1.82). Kruskal-Wallis-test showed a significant difference between groups ( $P < 0.05$ ). Epiphany showed significantly higher leakage values than GuttaFlow and RelyX Unicem (Mann-Whitney test,  $P < 0.05$ ).

**Conclusions** GuttaFlow exhibited the lowest leakage values. Epiphany allowed gross leakage to occur. RelyX-Unicem offers the potential for an adhesive root canal sealer, though modifications of handling characteristics are required.

### R32

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### Epiphany – influence of sealer placement and cone taper on microleakage

**Aim** To evaluate the influence of cone taper and placement technique on Epiphany root canal fillings.

**Methodology** Sixty extracted single-rooted teeth were randomly assigned to five groups of 12 teeth each and prepared

up to size 45, .04 taper with FlexMaster (VDW, Germany) and ProFile (Dentsply Maillefer, Switzerland) instruments. Canals were flushed with NaOCl during root canal treatment and finally rinsed according to manufacturer's instructions with a sequence of NaOCl 5%, EDTA 17% and sterile water (2 mL each). Canals were dried with absorbent paper points (PP). Root canal filling was carried out with the Epiphany (Pentron, Wallingford, CT, USA) obturation system (Epiphany primer, sealer, and cones) as follows (cone taper/placement technique): group 1 (size 40, .04 taper/PP), group 2 (size 40, .02 taper/PP), group 3 (no cone/lentulo). Twelve teeth were filled with size 40, .04 taper lentulo and sectioned horizontally in the middle part of the root. Then specimens were assigned to group 4 (apical part) and group 5 (coronal part). Another 12 teeth were obturated with size 40 .02 taper and assigned to group 6 (apical part) and group 7 (coronal part). Microleakage was evaluated using a dye penetration test (methylene blue 5%/centrifuging at 30 g for 3 min). After cross sectioning in steps of 1 mm specimens were evaluated for linear penetration depth under a stereo microscope (40×). The maximum possible leakage value was limited to 6 mm.

**Results** Linear dye penetration (mm) (means/SD): group 1 (4.42/1.83); group 2 (5.92/0.29); group 3 (2.75/1.48); group 4 (5.67/1.49); group 5 (5.58/1); group 6 (4.58/1.88); group 7 (3.83/1.75). Multifactorial ANOVA showed a significant influence ( $P < 0.05$ ) of both cone taper and combination of taper and placement technique, but no influence of the coronal/apical part of the root or the placement technique ( $P > 0.05$ ).

**Conclusions** Epiphany showed gross leakage in most groups.

### R33

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### Influence of sealer placement on apical sealer extrusion of two root canal sealers

**Aim** To evaluate the influence of sealer placement technique on apical sealer extrusion of GuttaFlow (Coltène/Whaledent, Germany) and AH Plus (Dentsply, Germany).

**Methodology** Sixty single-rooted teeth were randomly assigned to six groups ( $n = 10$ ) and the root canals prepared up to size 60, .02 taper. Apical patency was ensured with size 10 K-type reamers. After rinsing and drying root canals were filled with GuttaFlow (GF) or AH Plus (AH) using a single gutta-percha cone size 55, .02 taper. Freshly mixed sealers were inserted into insulin syringes (Terumo U-40 Insulin, Belgium) to inject well-defined amounts of 0.05 mL sealer per canal. Sealers were placed as follows (sealer/placement technique): group 1: GF/lentulo, group 2: GF/masterpoint, group 3: GF/cannula, group 4: AH/lentulo, group 5: AH/masterpoint, group 6: AH/cannula. For groups 1 and 4 the exact volume of sealer was placed onto a glass slab, picked up two times with a lentulo and placed into the root canals. For groups 2 and 5 the whole volume of sealer was picked up with the master cone prior to insertion into the root canal. For groups 3 and 6 the volume of sealer was directly placed into

the canals with the cannules of the GuttaFlow system. Extruded sealer was collected after setting and weighed using a precision balance (Sartorius CP124S, Sartorius, Göttingen, Germany).

**Results** The weight of apically extruded sealer was tendentially lower for GF (mg/SD): group 1: 3.62/2.04; group 2: 0/0; group 3: 1.43/2.24; group 4: 2.14/2.76; group 5: 0.07/0.22; group 6: 1.55/2.88. Univariate ANOVA displayed no significant influence of sealer type on apical extrusion ( $P = 0.42$ ) but a highly significant influence of placement technique ( $P < 0.001$ ). Insertion of AH displayed significantly less sealer extrusion within the masterpoint group compared to the other placement techniques (Mann-Whitney test,  $P < 0.01$ ). For GF sealer extrusion was merely significant between groups 4 and 5 (Mann-Whitney test,  $P < 0.05$ ).

**Conclusions** Placement of AH and GF by a syringe does not lead to greater weight of extruded sealer than with the use of a lentulo.

### R34

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### Adhesive coronal seal after different dentine pretreatment following use of AH plus sealer

**Aim** To find a suitable method for sealer removal prior to dentine bonding and restoration of the access cavity.

**Methodology** Fifty two teeth were selected for the study, 40 teeth with one root canal (four groups of 10 teeth) for a dye penetration test and twelve caries-free molars (two groups of 6 teeth) for a microtensile test. After preparation of standardized access cavities, root canals were prepared and then filled with AH plus and a single cone. Removal of excess sealer was performed with a foam pellet only (group 1), additionally by etching with 37% H<sub>3</sub>PO<sub>4</sub>-gel for 15 s (group 2), by preparation with an abrasive water-cooled diamond bur (group 3) or by preparation with a bur but after the placement of a temporary filling for 1 wk (group 4). An adhesive coronal filling on top of the root filling was performed with Syntac (Ivoclar-Vivadent, Liechtenstein) and Tetric flow (Ivoclar-Vivadent). For group 1 and 4, specimens for a microtensile test were prepared after the access cavity was filled with Tetric ceram (Ivoclar-Vivadent). Dye penetration was carried out by centrifugation for 3 min at 30 G within 5% methylene blue dye followed by examination under a stereo microscope (40×). Microtensile tests (group 1:  $n = 25$ ; group 2:  $n = 25$ ;  $\sim 1 \text{ mm}^2$ ) were performed with a universal testing machine (Zwick, Germany) at a cross head speed of 1 mm/min. Linear dye penetration was measured from crown to apex in teeth with root canal fillings and an adhesive coronal seal. Microtensile bond strength of the adhesive coronal seal was assessed only in groups 1 and 4. The statistical unit was the tooth.

**Results** Linear dye penetration (mm) was: (means/SD) group 1 (1.15/0.82); group 2 (0.17/0.6); group 3 (0/0); group 4 (0/0). Groups 2, 3 and 4 had significantly less leakage than group 1 ( $P < 0.01$ ; Mann-Whitney-test). In terms of micro tensile

strength, no significant differences could be detected [group 1 (5.18/0.99); group 4 (5.07/1.22) results in Mpa]. Thus, differences in sealing ability could not be attributed to differences regarding the strength of the dentine bonding.

**Conclusions** Acid etching or bur preparation of dentine prior to dentine bonding following the use of AH Plus sealer is recommended.

### R35

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### Long term evaluation of coronal leakage of root filled teeth, using different sealers

**Aim** To evaluate *in vitro* using a fluid filtration model coronal leakage along root fillings completed with 4 different root canal sealers.

**Methodology** The crowns of 100 extracted human maxillary central incisors were removed leaving roots 11 mm in length. After instrumentation by hand K-files using a stepback technique and smear layer removal, the 100 roots were divided randomly into four groups of 20 roots each, leaving 20 roots to serve as positive and negative controls. All groups were filled with cold lateral condensation of gutta-percha and a different sealer in each group: AH26 (Dentsply De Trey, Germany), Roth 601 (Roth International, USA), Epiphany (Pentron, USA), or RoekoSeal Automix (RSA) (Roeko, Germany). The specimens were stored in 100% relative humidity for 12 months and then measured for leakage with a fluid filtration model at a pressure of 0.3 atm. Results were expressed in microLitres/hour and categorized in a scale of no leakage (NL), slight leakage (SL) and gross leakage (GL), and then subjected to statistical analysis using Fischer's exact test.

**Results** For each sealer, the results were: AH-26: NL 2, SL 11, GL 7, Roth 601: NL 1, SL 5, GL 14, Epiphany: NL 8, SL 7, GL 5, RSA: NL 8, SL 5, GL 7. Statistical analysis revealed that when compared to AH-26, RSA ( $P < 0.05$ ) and Epiphany ( $P < 0.05$ ) were significantly different. No statistical differences were found between AH-26 and Roth 601.

**Conclusions** When a fluid filtration model was used for leakage evaluation, RSA and Epiphany leaked significantly less compared to AH-26 and Roth 601.

### R36

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### Leakage of bovine serum albumin in root canals filled with IRM and SuperEBA, with or without orthograde filling: an *in vitro* study using spectrophotometry

**Aim** To determine quantitatively the leakage of two apical root-end filling materials: SuperEBA (Stadent International,

UK) and IRM (DeTrey, Dentsply, Germany) in root canal samples with or without orthograde filling, by evaluating Bovine Serum Albumin (BSA) microleakage using a spectrophotometric method.

**Methodology** Thirty five single-rooted teeth were instrumented and divided into five groups. The apices of all teeth were resected. Root-end cavities in the samples from the first two groups, without orthograde root canal filling, were filled with SuperEBA (Group 1) and IRM (Group 2). The samples from the Groups 3, 4 and 5 were filled conventionally with gutta-percha and sealer, and after setting for 24 hours, the apices were resected. The teeth from Group 3 did not receive a root-end cavity preparation and filling. In the samples from Groups 4 and 5 root-end cavities were prepared and filled with SuperEBA (Group 4) and IRM (Group 5). Each sample was mounted in the glass vial and exposed to the BSA protein solution. Two sets of spectrophotometric measurements were conducted, after 7 and 60 days.

**Results** After 7 days microleakage was observed in only two specimens. After 60 days microleakage was recorded for all specimens. The greatest microleakage was observed in Group 2 ( $0.0041 \pm 0.0000011 \mu\text{g}$ ), then in Group 3 ( $0.0034 \pm 0.0000064 \mu\text{g}$ ) and Group 1 ( $0.0026 \pm 0.0000019 \mu\text{g}$ ). Samples from group 4 and group 5 leaked the least and to the same extent ( $0.0007 \pm 0.0000014 \mu\text{g}$ ). ANOVA analysis showed that there was a significant difference between the groups ( $F = 7.054$ ;  $p = 0.0000428$ ) and the Turkey's analysis showed statistically significant difference ( $P < 0.05$ ) between Groups 2 and 4, Groups 2 and 5, Groups 3 and 4, and Groups 3 and 5.

**Conclusions** Significantly less leakage occurred in samples filled with orthograde and root-end fillings than in the samples filled only with a orthograde approach and the samples with IRM root-end fillings.

### R37

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### Capillary Flow Porometry to assess the seal provided by root-end filling materials in a standardized and reproducible way

**Aim** To compare the root-end sealing ability of warm Gutta Percha (Obtura II, Obtura-Spartan, USA) +AH26 (Dentsply De Trey, Germany) (GP), Ketac-Fil Capsules (Espe, Germany) (KFil), Fuji IX Capsules (GC-Corporation, Japan) (FIX), Pro Root MTA ToothColoured Formula (Dentsply, USA) (MTA) and IRM Caps (Dentsply Caulk, USA) (IRM) in standard bovine root sections.

**Methodology** One hundred standard bovine root sections were prepared each 3 mm high with a diameter of 7 mm and an internal diameter of 2.5 mm. The sections were divided into 5 groups at random and each group was filled with a different root-end filling material. The filled sections were stored in an environment of 37°C and 95–100% humidity for 24 h, then exposed to distilled water and 24 h later submitted to capillary flow porometry (CFP) (PMI, USA) in order to assess the minimum, mean and maximum through-pore diameters of each root section. The results of the tests were statistically

evaluated by Kruskal-Wallis and Dunn tests. The level of significance was set at 0.05.

**Results** Significant differences were demonstrated for the mean and maximum pore diameters, not for the minimum pore diameters. For the mean and the maximum pore diameters, the results could be noted in the following order:  $\text{FIX} > \text{KFil} > \text{MTA} > \text{GP} > \text{IRM}$ . From the two by two analyses, it appeared there was a significant difference between FIX and the other materials for the mean diameters. For the maximum diameters, a significant difference between FIX and MTA, GP and IRM and between KFil and IRM was demonstrated.

**Conclusions** Under the conditions of this study, glass ionomer cements (GICs) demonstrated more leakage after 48 h than other root-end filling materials. It also appears there is a difference between GICs. Future measurements will have to show whether these results will remain equal as a function of time.

### R38

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### Comparison of sealing ability of three different root-end filling materials

**Aim** To compare *in vitro* the sealing ability of Mineral Trioxide Aggregate (MTA) (DeTrey Dentsply, Germany), Super EBA (Stident International, UK), and IRM (DeTrey Dentsply) in root end cavities prepared by Er:YAG laser.

**Methodology** After root canal instrumentation and filling the apices of sixty single-rooted teeth were resected. Root-end cavities 3 mm deep, were prepared with an Er:YAG laser. Laser beam parameters were: a pulse of very short duration (100  $\mu\text{s}$ ), energy of 280 mJ, and repetition rate of 10 Hz. Cavities within each group of 10 samples were filled with either mineral trioxide aggregate (MTA), Super-EBA or IRM. After 7 days in ink the teeth were cleared and the maximum degree of dye penetration for each specimen was measured with a stereomicroscope. The results were analysed statistically using Kruskal Wallis analysis of variance and Mann-Whitney *U* tests.

**Results** MTA ( $0.14 \pm 0.08 \text{ SD}$ ) had significantly less dye penetration ( $P < 0.05$ ) in comparison with Super EBA ( $0.41 \text{ mm} \pm 0.23 \text{ SD}$ ) and IRM ( $0.87 \pm 0.21 \text{ SD}$ ). There was no statistical difference between Super EBA and IRM.

**Conclusions** All three root end fillings allowed leakage to occur. Leakage with MTA was significantly lower than with Super EBA and IRM.



## R39

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### Radiopacity of root fillings in simulated canals: the effect of sealer

**Aim** To investigate the effect of root canal sealers on radiopacity of root fillings in simulated canals by means of digital radiography.

**Methodology** Thirty simulated root canals in transparent acrylic blocks were instrumented with HERO 642 (Micro-Mega, France) rotary instruments. Each canal was prepared to size 25 and a 4% taper. A single 4% tapered size 25 gutta-percha cone (Roeko, Germany) was inserted into each canal. Standardized images of the canals with an aluminium step-wedge were obtained using Digora (Soredex, Finland) storage phosphor plates. Then, three root canal sealers (RoekoSeal, Roeko, Germany; Diaket, 3 M Espe, Germany; Pulp Canal Sealer, Kerr, USA) were mixed according to the instructions of the manufacturers. The gutta-percha cones were completely coated with one of the sealers ( $n = 10$  per group) and placed in the identical canal to the full working length. Then, standardized images of the obturated canals were obtained. The mean gray values (MGVs) of gutta-percha cones with and without sealer at three different levels (1 mm, 6 mm, 11 mm from apex) was measured using Image Tool program (UTHSCSA, USA). Each MGV measurement was then converted to aluminium equivalent using step-wedge values. The differences between pre- and post-filling measurements were analyzed statistically with one-way analysis of variance and Bonferroni posthoc tests.

**Results** At the 1 mm- and 6 mm-levels, RoekoSeal caused a reduction in radiopacity of the filled canals ( $P < 0.05$ ). Radiopacity was increased from 0.3481 to 2.087 mm equivalent Al in Diaket and Pulp Canal Sealer specimens. There was no statistically significant difference between Diaket and Pulp Canal Sealer at any level ( $P > 0.05$ ).

**Conclusions** Root canal sealers can influence the radiopacity of root fillings. Radiopacity of sealer-gutta-percha combinations should be re-evaluated and standardized for improved clinical detection.

## R40

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### Apical control of gutta-percha root fillings using the ultrasonically energised thermocompaction technique

**Aim** To determine the influence of duration of activation, number of activations, and extent of penetration of an ultrasonically energised file on apical extension and voids in root fillings.

**Methodology** Root canals ( $n = 360$ ) were prepared to a standard .06 taper and randomly allocated to a control group

or one of eight test groups. Each group was divided between a general dental practitioner (GDP) and a postgraduate (PG); each filled 180 canals using cold lateral condensation (CLC, control,  $n = 20$ ) or an ultrasonically energised technique (UET, 8 test groups,  $n = 20$  each). An ultrasonically energised file was placed into gutta-percha once or twice, 1 or 3 mm from working length then either withdrawn immediately or after 2 seconds. The apical extent of root filling and voids were assessed by standard radiography and visual examination. The data were analysed using logistic regression models.

**Results** Roots with two canals were excluded, leaving 340 for analysis. Most root fillings (72.1%) were 'flush', 19% were 'short' and only 9% were 'long'. The GDP produced 'long' root fillings 36× more frequently than the PG ( $P < 0.001$ ). CLC resulted in 3.8× more 'flush' root fillings than UET ( $P = 0.014$ ). Penetration of the energised file closer to the canal terminus resulted in 1.8× more extruded or short root fillings ( $P = 0.017$ ). Voids were present in 54% of cases. The GDP produced 2× more voids than the PG ( $P < 0.001$ ). Deeper penetration of the energised file reduced the odds of prevalence of voids by 50% ( $P = 0.015$ ).

**Conclusions** 'Operator' and 'depth of energised file penetration' significantly influenced both apical extent of root filling and presence of voids, whilst the filling technique only affected the former.

## R41

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### Analysis of shrinkage of different gutta-percha treatments using an optical triangulation method

**Aim** To compare the shrinkage of two different gutta-percha treatments (alpha- and beta-gutta-percha of the Multi-Fill-System (Loser & Co GmbH, Germany) in comparison to commercial gutta-percha (Roeko, Germany).

**Methodology** The optical triangulation-method was used to assess shrinkage. Speckle Pattern Shearing Interferometry was used to test whether trapped air or material defects affected shrinkage. The three gutta-percha specimens were examined after heating to 90°C and cooling down to 35°C. Statistical analysis was performed by means of the Kruskal-Wallis test and the Wilcoxon test. Level of significance was set at  $P < 0.05$ .

**Results** The commercial gutta-percha shrank by 6.5%, while alpha-gutta-percha shrank by 7.2% and beta-gutta-percha by 7.3%. Shrinkage of the commercial gutta-percha was less pronounced and had a different temperature-dependency. In the range of 90°C to 55°C and 40°C to 35°C all 3 gutta-percha sticks shrank almost linearly. Between 55°C and 40°C the commercial gutta-percha plates did not show the rapid shrinkage noted with alpha- and beta-gutta-percha.

**Conclusions** Commercial gutta-percha had less shrinkage than the alpha- and beta-gutta-percha.

**R42**

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### Apical adaptation of root fillings completed using a soft resin canal filling system

**Aim** To compare apical quality of root fillings completed using cold lateral compaction (LC) and a soft resin canal filling system (Epiphany, USA).

**Methodology** Twenty four human single-rooted mandibular teeth were instrumented with System GT rotary files (Dentsply Maillefer, Switzerland) using a crowdown technique. Following removal of smear layer, teeth were randomly divided into two groups: Group 1: teeth were filled with the soft resin root filling system and Group 2 (control) with a cold lateral compaction technique using standard gutta-percha points. Epiphany root canal sealer was used in both groups. Horizontal sections were obtained from 1 mm up to 5 mm from the apex, using a low-speed saw. Digital colour images of sections were obtained at 40× under a stereomicroscope and transferred to an IBM compatible PC. Calculation of the canal area (in per cent) filled by material or sealer was performed by use of an image processor software (AutoCAD). The data were analyzed using unpaired Friedman Test and Mann Whitney-U tests.

**Results** There was no significant difference in apical filling adaptation between the groups.

**Conclusions** The new soft resin canal filling system was similar in terms of the apical adaptation of root filling in comparison with the conventional cold lateral compaction technique.

**R43**

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### Microleakage of root filled teeth after cyclic loading when restored with Glassix posts and metal crowns

**Aim** To evaluate *in vitro* the microleakage of root filled teeth after cyclic loading when restored with Glassix posts and metal crowns.

**Methodology** In 30 filled root canals of central maxillary incisors Glassix posts (Harald Nordin sa, Chailly/Montreux, Switzerland) were cemented with either Harvard (Richter & Hoffmann, Harvard Dental GmbH, Berlin, Germany), Fuji PLUS (GC Corporation, Tokyo, Japan) or Variolink II cements (Vivadent, Schaan, Lichtenstein) in three groups of ten canals each. The coronal restoration consisted of composite cores (Clearfil core, Kuraray, Osaka, Japan) and metal cast crowns. Specimens were embedded in acrylic resin and loaded on a

special testing machine. A load was applied at an angle of 135° to the long axis of the tooth, with forces oscillating from 0 to 35 N. Each specimen was exposed to 700 000 cycles through a period of 148 h. After performing cyclic loading, specimens were prepared for testing of microleakage. The acrylic bases were removed and the crowns were sectioned along with the composite cores and the coronal aspect of the Glassix posts to leave 15 mm long roots. Coronal microleakage was evaluated using a fluid transport system. The movement of an air bubble in a capillary glass tube connected to the apex of the experimental root section was measured over 5-min periods. Measurements were performed four times for each specimen and the mean values recorded. Analyses of variance were performed.

**Results** The highest values of microleakage (μL) occurred in the group cemented with Harvard cement (0.67), followed by Fuji PLUS (0.55) and Variolink II (0.22) cements. Results among the groups were significantly different ( $P < 0.05$ ).

**Conclusions** Canals with Glassix posts cemented with Variolink II cement had the least leakage after cyclic loading.

**R44**

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### A comparison of the penetration of three sealers into dentinal tubules: a SEM study

**Aim** To compare the penetration of Roeko Seal, AH Plus and Gutta Flow into dentinal tubules.

**Methodology** Fifteen single rooted extracted human teeth were used. The crowns of all teeth were sectioned and removed at the cemento-enamel junction. All canals were prepared chemo-mechanically up to a size 60 K-file and irrigated with 5.25 NaOCl, 30% citric acid and isopropyl alcohol. All teeth were randomly divided into 3 groups and filled with a single gutta-percha cone and sealer. In group 1 AH Plus (Dentsply DeTrey, Germany) was used as a sealer in groups 2 and 3 Roeko Seal (Coltene Whaledent, Germany) and Gutta Flow (Coltene Whaledent) were used respectively. The sealers were introduced into the root canals with a lentulo spiral. After setting the roots were grooved, longitudinally split and examined under a scanning electron microscope. The penetration of the sealers into the dentinal tubules was examined 3 mm, 6 mm and 9 mm from the root apex at 1500 and 3000 magnification. The focus of observation was the interface between the dentine and the sealing material. The numbers of examinations with positive sealer penetration were noted for each sealer and compared using Kruskal-Wallis and Mann-Whitney tests.

**Results** Statistical analyses revealed that in comparison with AH plus, the other two sealers had significantly more sealer penetration ( $P < 0.05$ ). There was no significant difference in sealer penetration between Roeko Seal and Gutta Flow.

**Conclusions** AH plus sealer had less penetration into dentinal tubules than Roeko Seal and Gutta Flow.

## Research Posters – Materials Science

### R45

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#### Physical characteristics and surface analysis of grey and white MTA and Portland Cement

**Aim** To analyze and compare the pH value, the conductivity, the particle size distribution and the surface characteristics of grey and white Mineral Trioxide Aggregate (ProRoot MTA, Dentsply, USA) and Portland Cement (PC).

**Methodology** pH value and conductivity were measured in suspensions produced after mixing material specimens that were allowed to set for 4 h with 50 mL of distilled water. Initial measurements were taken and subsequently repeated after 24 h, 48 h and 7 days. Particle size distribution of white MTA powder was measured using a CILAS device (Compagnie Industrielle de Lasers, France). This equipment counts the percentage of particles of different size in the material. Surface characteristics of material specimens, stored at 100% humidity and 37°C, were analyzed by profilometry (Diavite DH-5, Switzerland) at time intervals of 72 h, 7, 15 and 30 days. The values of Ra were calculated and specimens observed under SEM.

**Results** Grey MTA and PC had very similar pH and conductivity values at all time intervals. These values were slightly higher than those of white MTA and PC, which were themselves very similar. Particle size distribution analysis showed that 100% of the particles of white MTA had a diameter smaller than 24 µm. Profilometric analysis did not show any difference between the materials at any time interval. All of the specimens had a considerable surface roughness and the mean value of Ra (in µm) ranged between 1.71–2.13 for white MTA, 1.87–2.24 for white PC, 1.63–2.06 for grey MTA and 1.56–2.13 for grey PC. However, SEM analysis revealed differences between the materials.

**Conclusions** MTA has similar physical and surface characteristics to Portland Cement. This is especially true for materials of the same colour. However, SEM analysis and particle size distribution analysis revealed an important difference between the materials: MTA is composed of much finer particles than Portland Cement.

### R46

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#### The effect of condensation pressure on selected physical properties of mineral trioxide aggregate (MTA)

**Aim** To examine the effect of condensation pressure after mixing on the surface hardness, micro-structure and compressive strength of MTA.

**Methodology** Tooth-coloured mineral trioxide aggregate (ProRoot, Dentsply, USA) was mixed according to the manufacturer's instructions, divided equally by weight and packed into cylindrical polycarbonate tubes. Six groups each of 10 specimens were prepared using pressures of 0.06 MPa, 0.44 MPa, 1.68 MPa, 3.22 MPa, 4.46 MPa and 8.88 MPa respectively. Pressure on each specimen was applied for 1 minute using a custom-made device. Condensed samples were retained in the polycarbonate tubes and kept in 100% humidity at room temperature for 4 days. The surface hardness of each specimen was measured using Vickers hardness. Data were subjected to one-way ANOVA. The micro-structure was analysed using a SEM after sectioning specimens with a scalpel.

**Results** A trend was observed for higher condensation pressures to produce lower surface hardness values. A condensation pressure of 8.88 MPa produced specimens with statistically significantly lower values in terms of surface hardness than other groups ( $P < 0.001$ ). A condensation pressure of 1.68 MPa conferred the maximum compressive strength; however, it was not statistically different from the other groups. Higher condensation pressure resulted in fewer voids within the specimens, when sectioned and analysed with SEM, however, un-reacted MTA particles scattered in a non-uniform matrix were present. In specimens prepared with lower condensation pressure distinctive crystalline structures embedded within a more uniform matrix were seen.

**Conclusions** Variable factors may affect the strength and hardness of MTA and optimal hydration of the material crystals during setting may improve its physical properties.

**Acknowledgements** Mrs. Sadighe Salariye, Mrs. Wendy Rowe, Mr. Brian Western, Mr. D C Stone, Mr. P J Milward.

**R47**

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### The effect of pH on the surface hardness and micro-structure of mineral trioxide aggregate

**Aim** To evaluate the effect of pH on the surface hardness and microstructure of MTA.

**Methodology** Tooth-coloured mineral trioxide aggregate (ProRoot, Dentsply, USA) was mixed according to the manufacturer's instructions, divided equally by weight and packed into identical cylindrical polycarbonate tubes. Four groups each of 10 specimens were prepared using a pressure of 3.22 MPa applied for 1 minute in a custommade device. Condensed samples were retained in the polycarbonate tubes and each group was exposed for 4 days to butyric acid that had been buffered at either pH 4.4, 5.4, 6.4 and 7.4 respectively. The surface hardness of each specimen was measured using Vickers hardness. Data were subjected to one-way ANOVA. The microstructure was analysed using a SEM after sectioning specimens with a scalpel.

**Results** The highest surface hardness values were observed with pH 7.4 and the values decreased through to pH 4.4. The difference between the Vickers hardness values were statistically significant ( $P < 0.001$ ). Specimens kept in contact with pH 4.4 butyric acid had un-reacted MTA particles scattered in a non-uniform matrix whereas those kept in contact with pH 7.4 had distinctive crystalline structures embedded within a more uniform matrix.

**Conclusions** Surface hardness and setting reaction of MTA is impaired in an acidic environment.

**Acknowledgements** Mohammad Saeed Sheykh Rezaeer, Dr Gabriel Adusei, Ms. Wendy Rowe, Mr. Brian Western, Mr. Christopher Fenton.

**R48**

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### Chemical analyses of different gutta-percha products used for cold and warm filling techniques

**Aim** To determine the chemical composition of eleven commercially available gutta-percha materials for root canal filling and evaluate the differences between cold and warm gutta-percha products.

**Methodology** Eleven gutta-percha formulations were chosen for chemical analyses: Gutta-percha points (Dentsply Maillefer, USA), Thermafil (Dentsply Maillefer), Autofit for warm techniques (Analytic Endodontics, USA), Autofit for greater taper (Analytic Endodontics), Obtura (Obtura, USA), Ultrafil white-green-blue (Coltene/Whaledent, USA), Hygenic points (Col-

tene/Whaledent), Successfil (Coltene/Whaledent) and Softcore (Septodont, UK). The organic fraction (gutta-percha polymer and wax/resin) was separated from the inorganic fraction (zinc oxide, barium sulphate) by dissolution in chloroform. Gutta-percha polymer was precipitated with acetone and the remaining soluble material in acetone (wax/resin) was determined after evaporation. For the determination of the inorganic fraction new samples of the same materials were used. The samples were calcinated in an electrothermal oven at 550°C to eliminate organic compounds. The ashes were washed with nitric acid to dissolve zinc oxide. Zinc oxide was determined by atomic absorption spectroscopy.

**Results** The minimum and maximum percentage of each component was as follows: for gutta-percha the lowest percentage (16%) was found in Ultrafil white, while Autofit for warm technique had the highest percentage (22%). The wax/resin percentage was 1% for Dentsply points and 4% for Obtura. The minimum percentage of zinc oxide (50%) was found in Softcore and the maximum (78%) in Dentsply points. Dentsply points also had the minimum percentage (3%) of barium sulphate, while Softcore had the maximum (27%).

**Conclusions** New gutta-percha formulations showed great chemical heterogeneity among different manufacturers and among different products of the same manufacturer used for cold and warm techniques.

**R49**

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### Contact angles of various endodontic sealers

**Aim** To measure the contact angles of two conventional endodontic sealers, Roth (Roth International, USA) and AH26 (Dentsply De Trey, Germany), and two silicone based sealers, Roekoseal (Roeko, Germany) and Gutta-Flow (Coltene/Whaledent, Germany) on dentine and gutta-percha surfaces.

**Methodology** Controlled volume droplets of each sealer were placed onto 10 dentine disks and onto 10 gutta-percha slabs. A photo was taken of each specimen under standard conditions after 5 min and after 60 mins. The contact angle was measured mathematically with a goniometer attached to a computerized system. The contact angle was calculated from the base width and height of the droplet.

**Results** The mean values of contact angle onto dentine disks for the first observation period (5 mins) were for AH26 = 14.5, Roth = 11.1, Roekoseal = 44.6, Gutta-Flow = 45.1. The mean values of contact angle onto gutta-percha slabs for the first observation period were for AH26 = 18.2, Roth = 16.0, Roekoseal = 45.5, Gutta-Flow = 41.4. Lower mean values of contact angle for all sealers were obtained after 60 mins. Statistical differences were not found between the two conventional sealers and between the two silicone based sealers for the two observation periods. However, statistical differences were found between the conventional and silicone based sealers for the two observation periods ( $t$ -test,  $P < 0.001$ ).

**Conclusions** Conventional sealers (AH26, Roth) had lower contact angles than the silicone based sealers (RoekoSeal, Gutta-Flow).

## R50

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### Physical properties of newly developed root canal sealers

**Aim** To investigate selected physical properties of eight root canal sealers.

**Methodology** The radiopacity, film thickness, flow and compressive strength of AH Plus (DeTrey Dentsply, Germany), Epiphany (Pentron, USA), EndoREZ (Ultradent, USA), RC Sealer (Sun Medical, Japan), Acroseal (Septodont, France), Apexit (Vivadent, Leichtenstein), RoekoSeal (Roeko, Germany) and GuttaFlow (Coltene/ Whaledent, Germany) were evaluated. Radiopacity and film thickness measurements were performed in accordance with the International Organization for Standardization (ISO) standard 6876-2001. Flow measurements were performed in accordance with ISO/DIS 6876-1984. For compressive strength measurements, cylindrical test specimens, 6 mm high and 4 mm in diameter were produced in stainless-steel moulds and tested in a universal testing machine after 1, 4, 18, 24 and 48 h of incubation.

**Results** The radiopacity ranged from 9.9 to 4.5 mm of aluminium and decreased in the following order: AH Plus > Epiphany > EndoREZ > RC Sealer > RoekoSeal > GuttaFlow > Apexit > Acroseal. The film thickness ranged from 3 µm to 43 µm and increased in the following: EndoREZ < RoekoSeal < Apexit < Epiphany < AH Plus < GuttaFlow < Acroseal < RC Sealer. The flow of the sealers ranged from 44.67 to 22.1 mm and decreased in the following order: RoekoSeal > Epiphany > Apexit > Acroseal > GuttaFlow > AH Plus > EndoREZ > RC Sealer. A wide variation in compressive strength was noticed ranging for fully set test pieces from 6.59 MPa (Apexit) to 336.39 MPa (EndoREZ). Except Epiphany, which reached maximum strength almost as soon as the material was hard enough to be removed from the mould, other sealers showed increasing compressive strength values for periods up to 2 days. GuttaFlow, RoekoSeal and RC sealer were unsuitable for testing compressive strength.

**Conclusions** The endodontic sealers had satisfactory physical properties according to the ISO standards 6876-1984 and 2001.

**Acknowledgements** The financial support of The Research Council of Norway and NIOM-Scandinavian Institute of Dental Materials.

## R51

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### Influence of occlusal contacts on stress distribution in two-rooted teeth restored with aesthetic endodontic posts

**Aim** To investigate the stress distribution of a new composite post and to compare the changes in the stresses in normal occlusion and in malocclusion.

**Methodology** The 3D finite element method (FEM) was used to perform the stress analysis of the two rooted first maxillary premolar restored with glass fibre posts. Composite resin was used as the core material and a full porcelain crown covered the preparation. Four noded tetrahedral elements were applied in the description of the tooth morphology, resulting in 1 684 512 elements and 246 510 nodes with 739 530 degrees of freedom. A total force of 200 N was applied.

**Results** In the case with normal occlusion, stress distribution was mainly compression in its nature (from -4.7 to -230 MPa), except in the fissure where stress was tensile (+2.9 MPa). In the case with malocclusion, tensile stress was generated on the cervical areas of both the sound tooth (+74 MPa) and restored tooth (+6.5 MPa). At the root furcation, tensile stress appeared only on the restored tooth (+4.7 MPa).

**Conclusions** In the case of malocclusion, the post and core modified the stress distribution at the root furcation, therefore increasing the possibility of root fracture.

## R52

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### The effect of polyethylene fibre on fracture resistance of immature maxillary central incisors

**Aim** To evaluate *in vitro* the reinforcement effect of a resin cement with or without leno woven ultra high modulus polyethylene fibre on fracture resistance of immature maxillary central incisors.

**Methodology** Forty sound extracted human maxillary central incisors were used in this study. In Group 1 (negative control,  $n = 5$ ), access cavities were prepared and restored with composite resin. Teeth in Groups 2 to 5 were prepared with burs to simulate the thin dentinal wall of immature teeth throughout their length. The groups were restored as follows: Group 2 (Positive control,  $n = 5$ ): access cavities were restored with composite to the level of the CEJ, Group 3 ( $n = 10$ ): the canals were obturated with AH-Plus root canal sealer (Dentsply DeTrey, Germany) and thermoplasticized gutta-percha (Obtura, Unitek Corp., USA), Group 4 ( $n = 10$ ) and 5 ( $n = 10$ ): Apical 3 mm of the canals were obturated with AH-Plus root canal sealer and thermoplasticized gutta-percha. The rest of

the canal system was filled with Panavia-F (Kuraray Co. Japan) in Group 4 and Panavia-F resin was reinforced with a polyethylene fibre (Ribbond, USA) in Group 5. After restoring the access cavities with composite. The specimens were stored in 100% humidity at 37°C for 24 h and then subjected to compressive force using an Instron testing machine at a crosshead speed of 0.5 mm/min until fracture. The data was recorded as Newton (N) and subjected to analysis of variance (ANOVA) and Tukey post-hoc test.

**Results** The mean load necessary to fracture the samples in each group were (in N): Group 1: 1019.22 ± 64.00a, Group 2: 391.86 ± 23.76b, Group 3: 398.99 ± 35.75b, Group 4: 781.54 ± 50.99c, Group 5: 925.71 ± 54.96d. Different letters show significantly different groups ( $P < 0.05$ ).

**Conclusions** Use of resin cement in root canals of immature teeth increased fracture strength, however, the highest fracture strength values were obtained with reinforcement of resin cement using a polyethylene fibre.

### R53

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### Evaluation of the fracture resistance of root filled teeth restored with different restorative materials and post systems

**Aim** To compare the fracture resistance of teeth restored with new generation composite resins and two post systems following root canal treatment.

**Methodology** Seventy maxillary premolar teeth were used. The root canals were enlarged with K-files to size 50. A standard flare was produced using sizes 2–5 Gates Glidden drills. Irrigation was performed using a 2.5% NaOCl solution. The root canals were filled with gutta-percha and AH26 using the lateral condensation technique. MOD cavities were prepared in each tooth so that the thickness of the buccal wall measured 2 mm at the occlusal surface and 3 mm at the CEJ. The teeth were randomly allocated into 7 groups each comprising 10 teeth. Each group were restored with the following materials: Group 1: Control-intact teeth, Group 2: Prepared and filled, unre-stored, Group 3: Packable composite resin (Solitaire2 + I Bond), Group 4: Packable + flowable composite resin (Solitaire2 + Flowline composite + I Bond), Group 5:Ormocer (Admira + Admira Bond), Group 6: Glass fibre post system (Everstick Post + Renew + One Step Bond), Group 7: Zirconia post system (Cosmo Post + Renew + One Step Bond). Using an universal test device a force of 1 mm/min at an angle of 150 degrees between tooth and material was applied to each specimen until failure. The Results were evaluated statistically using Kruskal Wallis.

**Results** Intact teeth were the most resistant; teeth prepared and unrestored had the poorest resistance values. Ormocer and the post groups were significantly more resistant ( $P < 0.05$ ) than Solitaire2. No significant difference was found between Solitaire2 with or without flowable resin. Also no significant difference was found between the two post groups ( $P > 0.05$ ).

**Conclusions** In this laboratory study flowable resin did not increase resistance to fracture whereas the glass fibre zirconia post systems combined with composite resin enhanced resistance. The fracture values of Solitaire2 and Solitaire2 with flowable composite may be a reflection of the one-bottle dentine bonding system I Bond.

### R54

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### A temporary filling material may cause cusp deflection, infractions and fractures in endodontically treated teeth

**Aim** To test the hypothesis that Coltisol F (Coltène/Whaledent Inc., Switzerland) might cause infraction and cusp fracture due to material expansion.

**Methodology** Thirty two extracted human molars were root filled and prepared with MOD cavities with or without undercuts. The specimens were filled proximally with glass ionomer cement and then occlusally with either Coltisol F or zinc oxide eugenol cement. The tooth specimens were kept in water at 37°C for a period of 20 days, and every second day, the inter cusp distance (ICD) of each specimen was measured in a travelling microscope, and the number of infraction lines as well as fractures were noted.

**Results** The number of infraction lines increased in teeth filled with Coltisol F. Between day 8 and 16, seven out of 16 teeth filled with Coltisol F had fractures and exhibited a mean increase in ICD of 316 ± 156 µm. Teeth filled with ZOE did not show an increase in number of infraction lines or in ICD, and none had fractures.

**Conclusions** The hygroscopic expansion of Coltisol F in a cavity may lead to cusp deflection, infraction development and fracture. The material is not recommended for temporary filling except for a few days.

### R55

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### Flexural properties of human dentine: an *in vitro* study

**Aim** To evaluate the mechanical properties of human root dentine under flexural stresses.

**Methodology** Standardized dentine bars ( $n = 20$ ) with 1.5 mm × 1.5 mm sides and at least 10 mm in length were obtained from intact, single rooted, completely formed human premolars, freshly extracted for orthodontic reasons. The specimens were selected from a pool of dentine bars evaluated for cracks and defects under stereomicroscope at 30× and with µCT analysis (Skyscan 1072, Assing s.p.a.; Belgium). The

specimens were stored in normal saline until testing and then loaded to failure in a three-point bending test with an electronic dynamometer (Lloyd Instruments Ltd, UK). The three-point bending test according to the ISO 10477 standard (span 10.0 mm, crosshead speed 1.0 mm/min, cross-sectional diameter of loading tip 2 mm) was used to measure the flexural modulus and flexural strength of the dentine bars. The load-deflection curves were recorded with PC-software (Nexygen Mt v4.5, Lloyd Instruments Ltd, UK).

**Results** The flexural modulus registered for human root dentine was  $18.58 \pm 3.7$  GPa and the flexural strength was  $216.38 \pm 45.84$  MPa.

**Conclusions** Mechanical properties of human root dentine under flexural stresses obtained in this study are similar to those obtained in other studies with different techniques (Young's Modulus), commonly considered the reference parameter in the evaluation of the mechanical properties of materials as posts. The values are similar to those reported for the most commonly used fibre reinforced root canal posts.

## R56

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### Effect of heat on the physical properties of dentine treated with saline or 2.5% NaOCl

**Aim** To evaluate the effect of heat on physical properties [flexural strength (FS), maximum strain (MS), storage modulus (SM) and tan delta (TD)] dentine bars treated with either saline or 2.5% sodium hypochlorite.

**Methodology** Eighty-six dentine bars fabricated from different teeth were randomly distributed to 8 test groups and stored in 0.9% saline. Groups 2, 4, 6 and 8 were treated with 2.5% NaOCl for a period of 20 minutes. Groups 1, 3, 5 and 7 were untreated. Groups 1 & 2 ( $n = 30$ ) and groups 3 & 4 (which were heat treated to 200°C for 10 min and re-hydrated in 0.9% saline;  $n = 30$ ) were assessed for FS and MS using an Instron loading machine. Groups 5 & 6 ( $n = 20$ ) were subjected to Dynamic Mechanical Analysis (DMA) to assess SM and TD, at room temperature. DMA was repeated after heating groups 5 & 6 to 200°C and repeated for a second time following rehydration of groups 5 & 6 in 0.9% saline. Groups 7 & 8 ( $n = 6$ ) were subjected to DMA continuously between 25°C–185°C. Raman spectroscopy was performed on a single dentine bar from each of the groups 1–4.

**Results** There were no significant differences in mean FS or MS for groups 1 & 2 (no heat treatment) and groups 3 & 4 (subjected to heat). DMA of group 6 (subjected to 2.5% NaOCl) revealed a significant decrease in TD ( $P < 0.05$ ) after heat treatment but no change in SM. Group 5 (untreated) showed a significant ( $P < 0.05$ ) increase in SM and concurrent decrease in TD following heat treatment.

**Conclusions** Heating dentine up to 200°C is unlikely to affect its flexural strength or maximum strain irrespective of prior treatment with 2.5% NaOCl. The storage modulus is also unlikely to be affected by heat provided that re-hydration occurs. Storage modulus varied with the temperature of dentine when exposed to saline but not when exposed to NaOCl.

## R57

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### Effect of irrigant solutions on the microhardness of root dentine

**Aim** To evaluate the effects of NaOCl, EDTA, EGTA, EDTAC, and Tetracycline-HCl solutions on the microhardness of root dentine.

**Methodology** Thirty single-rooted teeth were sectioned longitudinally into 2 equal halves. Each half was embedded in acrylic blocks and metallographic grinding and polishing completed. The 60 specimens were randomly divided into 6 equal groups. Vickers hardness of root dentine in the middle third was measured using a microhardness tester with a 200-g load with a 20 second contact time. The specimens were then irrigated for 1 minute using 2.5% NaOCl, 17% EDTA, 17% EGTA, 15% EDTAC, Tetracycline-HCl or distilled water. The microhardness procedure was repeated. The percentage differences before and after irrigation were analyzed statistically with ANOVA and Kruskal-Wallis tests.

**Results** All the irrigant solutions decreased significantly the microhardness of root dentine ( $P < 0.05$ ). The difference in microhardness when EDTA was applied was significantly more than the other groups ( $P < 0.05$ ). Although irrigation with Tetracycline-HCl, EDTAC and EGTA reduced dentine hardness compared to NaOCl irrigation, there was no statistically significant difference between them. EGTA application reduced microhardness significantly more than the distilled water ( $P < 0.05$ ).

**Conclusions** EDTA irrigating solution reduced significantly the microhardness of root canal dentine compared to the other solutions.

## R58

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### In vitro examination of the effect of bleaching agents on dentine microhardness and atomic structure

**Aim** To evaluate the effects of non-vital bleaching agents on dentine microhardness and to investigate the changes created by these agents on dentine surfaces using Quartz Crystal Non-Contact Atomic Force Microscopy (NC-AFM).

**Methodology** The roots of twenty extracted mandibular molar teeth were removed at the cemento-enamel junction, and the crowns sectioned buccolingually and mesiodistally into 4 sections to measure microhardness of the dentine in such a manner that control and test sections were located on the same tooth. In each tooth one section was used as the control. On the remaining dentine sections either 10% hydrogen peroxide (HP), 30% HP and a paste of sodium perborate (SP) mixed with distilled water were applied. Dentine microhardness of all specimens were measured before and after application of bleaching agents. NC-AFM evaluation:

Dentine sections were prepared in the same manner. Initially, all the surfaces covered with smear layer were examined by NC-AFM. After the removal of smear layer 10% HP, 30% HP and SP were applied on the same smear free surfaces for 15 min. NC-AFM examinations were made before and after application of the test materials.

**Results** Statistical analysis showed that HP solutions significantly reduced dentine microhardness when compared with SP ( $P < 0.01$ ); but the most significant difference was produced after application of 30% HP. NC-AFM images and depth profiles demonstrated that both HP solutions deepened the grooves; their deepening effect increased with increasing concentration. SP caused minimal microstructural changes in dentine that made the dentine surfaces relatively smooth.

**Conclusions** High concentrations of HP caused a significant decrease in dentine microhardness and caused microstructural changes. SP paste created less damage.

## R59

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### SEM investigation of the MTA/Composite interface in simulated immature teeth

**Aim** To evaluate the quality of the interface of MTA (Dentsply Tulsa, USA) and various types of composites in simulated immature teeth.

**Methodology** Seventy single rooted extracted teeth were selected, radiographed and their canals enlarged using a crowdown technique with Gates Glidden burs and K-type files. The apical 2 mm of each root were then removed and the apical portion of the canals were enlarged using K3 (Sybron-Endo, USA) in a retrograde approach to simulate an open apex. Teeth were randomly divided into 5 experimental groups and 2 controls. The experimental groups were filled using MTA to a depth of 4 mm at the apex followed by 5 different composite systems each used according to the manufacturer's instructions. Group 1 had a self cured composite; group 2 a base-increment self cured composite; group 3 a dual cured luting composite; group 4 and 5 had two different light cured composites. Teeth in group 6 had gutta-percha and sealer placed on the MTA and in group 7 the canals were filled completely with MTA (controls). Teeth were embedded in resin, longitudinally sectioned and processed for SEM to evaluate the interface between the dentinal wall, the MTA and the composite restorations. A scoring system was used and the Kruskal Wallis one-way ANOVA and the Mann-Whitney tests were applied.

**Results** The best interface was observed between the MTA, the light cured traditional composites and dentine; the results were statistically relevant ( $P < 0.05$ ).

**Conclusions** In this laboratory study the best combination of MTA and composite for the filling of immature roots was obtained with light cured traditional composite.

## R60

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### A new device for cyclic fatigue testing of NiTi rotary endodontic instruments

**Aim** To evaluate a new device for cyclic fatigue testing of NiTi rotary endodontic instruments that permits the use of precise parameters for canal angle and radius of curvature.

**Methodology** The device consisted of a frame that supports a handpiece and a stainless steel block with artificial canals. Each artificial canal is matched for each instrument size and taper, in such a way that provides a precise trajectory pathway for the instruments. The number of cycles to failure (NCF) was compared in the new device (artificial canals calibrated and with tapered shape – Group A) and in 18-gauge stainless steel needles with an internal diameter of 0.83 mm (artificial canals not calibrated and with no tapered shape – Group B). The geometrical parameters were radius of curvature = 2 mm, angle of curvature = 60°; the point of maximum curvature was 6 mm from the tip of the instruments. Ten instruments ProFile (Dentsply Maillefer, Switzerland) taper .06 size 25 were tested for each Group. An electric handpiece was configured to rotate at 300 rpm. The NCF was calculated from the rpm data by multiplying the rpm by the time of failure. Data were analyzed by one way analysis of variance (ANOVA) and *t*-test using a 95% confidence level.

**Results** The mean NCF was significantly lower ( $P < 0.005$ ) for Group A (NCF =  $79.5 \pm 8.5$ ) than for Group B (NCF =  $346.5 \pm 24.7$ ), despite the same geometrical parameters being used.

**Conclusions** If the artificial canal is not identical in shape and size to the instruments, their movement is less predictable and could influence the results of the cyclic fatigue test. The new device improves the reliability of cycling fatigue tests.

## R61

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### Root canal morphology of mandibular central and lateral incisors after root-end resection

**Aim** To evaluate macroscopically the root canals of mandibular incisors after root-end resection in horizontal cross-sections in the apical region, and on longitudinal sections.

**Methodology** The study included 120 extracted mandibular central and lateral incisors. After determination of the working length, the canals of all teeth were chemomechanically prepared 0.5 mm short of the apex foramen to size 40 K-file with a stepback technique and then filled with laterally condensed gutta-percha (Roeko, Germany) and AH Plus sealer (Dentsply DeTrey, Germany). After resecting horizontally 3 mm of each root apex, the canal shapes on the remaining root-end were evaluated. Longitudinal sections of the teeth



were made in the labio-lingual direction and the morphology of the entire canal length assessed.

**Results** The following shapes of the canals in the apical region were observed on the horizontal cross-sections: I – a single round canal in 59% of the cases; II – two separate round canals with possible isthmus occurrence – 12%; III – a single canal with isthmus running in the labial and lingual directions – 29%. Five types of canals were found on the longitudinal sections: I – a single canal – 59%; II – two separate canals – 9%; III – two canals, main buccal and the second one lingual located 1–2 mm beneath the CEJ, fusing into one in the apical region or terminating with two separate orifices – 16%; IV – one canal dividing into two separate ones and fusing again into one – 13%; V – one canal dividing into two separate ones – 3%.

**Conclusions** In the apical region after root resection a round single canal was observed on horizontal cross-sections in the majority of mandibular incisors (59%). On longitudinal sections a single root canal was detected in 59% of the cases.

## R62

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### Solubility of AH 26 at different consistencies using Endosolv E and R root filling removers

**Aim** To compare the weight loss of AH 26 (Dentsply De Trey, Germany) at two consistencies in two different solvents: Endosolv R (Septodont, France) and Endosolv E (Septodont).

**Methodology** Forty standardized stainless steel ring moulds were filled with epoxy resin based root canal sealer AH 26 in either the normal ( $n = 20$ ) or a viscous consistency ( $n = 20$ ) after which the samples were allowed to set for 24 h at 37°C and 95% relative humidity. For the normal viscosity AH 26 was mixed at a one to one ratio as prescribed by the manufacturer, for the viscous consistency extra powder was added (1.25 : 1). The groups were each divided in four groups ( $n = 5$ ), and each group submitted to immersion in a solvent according to one of the following procedures: (i) Endosolv R for 1 minute, (ii) Endosolv R for 5 minutes, (iii) Endosolv E for 1 minute, (iv) Endosolv E for 5 minutes. The samples were dried for 24 h at 37°C. Before and after immersion and drying, the samples were weighed three times and the difference of the mean weights was calculated. The mean percentage loss of weight was calculated for each sample. The results were evaluated statistically using Kruskal-Wallis and Mann-Whitney tests. Level of significance was set at 0.05.

**Results** Solubility of AH 26 – normal was significantly higher than that of AH 26 – viscous ( $P < 0.001$ ). Endosolv E caused significantly greater dissolution than Endosolv R ( $P < 0.005$ ). There was no significant difference between 1 and 5 minutes immersion time.

**Conclusions** Although Endosolv E is believed to remove zinc oxide eugenol types of sealers and Endosolv R to remove phenolic resin type of sealers, under the conditions of this study, Endosolv E was a more effective solvent of AH 26. Increasing the powder-liquid ratio made AH 26 more resistant to dissolution.

## Research Posters – Pathology

### R63

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### Presence of T cell subpopulations in apical granulomas and apical cysts

**Aim** To investigate immunohistochemically the presence of T helper/inducer (Thi) and T cytotoxic/suppressor (Tcs) lymphocytes in apical granulomas and cysts.

**Methodology** Periapical lesions which were to be treated surgically were used in this study. Twelve histologically identified granulomas and nine apical cysts were immunohistochemically stained employing the immunoperoxidase technique. Positive cells were counted under the light microscopy and analysed statistically.

**Results** In both cysts and granulomas, the mean number of Thi cells was more numerous than Tcs cells, but with no statistically significant difference. The Thi/Tcs ratios for cysts and granulomas were 1.09 ( $\pm 0.94$ ) and 1.14 ( $\pm 0.74$ ) respectively, without significant difference between the lesions.

**Conclusions** There was no predominating presence of a certain type of T cell subpopulation associated with the periapical lesion type. The Thi/Tcs cell ratios found in both granulomas and cysts may indicate that both lesions were in chronic phase.

### R64

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### Pulpoperiapical osteosclerosis: prevalence in contemporary and archaeological populations

**Aim** To describe pulpoperiapical osteosclerosis in sample populations from the same geographical region (southern France) over a period of 4 millennia through an epidemiological study.

**Methodology** Sample populations were selected on the basis of jawbone condition in terms of clinical and/or anthropological data. The oldest sample included 50 individuals from the chalcolithic hypogea (–2000 BC) in Roaix (Vaucluse). The historic sample (IVth to XVth century) included 250 individuals from the Notre-Dame-du-Bourg grave site (Alpes de Haute Provence). The contemporary sample included 223 individuals recruited from various dental practices in the Gard Department, France. Data for all individuals were obtained by digital radiographic imaging of the jawbones using an image processing software application (Sensor CCD Visualix, Gendex Dental Systems, Italy). Differences in lesion prevalence were analysed in terms of

individual and dental features (Period, age, and gender; jawbone, dental group, and tooth type). Statistical analysis was performed using the Chi Square tests and Fisher exact test.

**Results** The prevalence of lesions showed an increase between archaeological samples and then decreased in the contemporary sample ( $P < 0.001$ ). Regardless of the time period lesions were more likely to be found on the mandible ( $P < 0.001$ ) and to involve the molar group ( $P < 0.001$ ), especially the first molars ( $P < 0.01$ ). Age and gender did not appear to be significant factors.

**Conclusions** Comparison with other archaeological samples is not possible since there are no previous reports on pulpoperiapical osteosclerosis. Most of the findings in the contemporary sample are in agreement with those reported elsewhere. The decrease in the prevalence of these lesions could be attributable to improvement in living conditions and to the development of dental care. This has not been the case for most other apical pathosis.

### R65

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### Influence of electronically controlled periapical instrumentation on periapical healing

**Aim** To study the influence of electronically controlled periapical instrumentation on the healing of chronic periapical lesions in the teeth of dogs.

**Methodology** After inducing periapical lesions in six mongrel dogs for 35 days, root canal treatment was performed using two different protocols. In group 1, 'intracanal instrumentation' (3 dogs – 17 canals), canals were instrumented to the apical delta with crown-down technique using ProFile (Maillefer Dentsply, Switzerland) instruments and then filled with Thermafil obturators (Maillefer Dentsply, Switzerland). In group 2, 'electronically controlled periapical instrumentation' (3 dogs – 18 canals), following the same procedure as in group 1, the apical delta was perforated and additional instrumentation was performed using a hand H-file to the length determined by a resistance type apex locator (EED-11, Struja, Croatia), on average 1.05 mm beyond the apex. Canals were filled as in the first group, 2 mm shorter than the apical foramen, where a new artificial apical constriction was created. Thirty-five days after filling, undemineralized sections 5–7  $\mu$ m thick were stained with Toluidine blue and analyzed using light microscopy. Histomorphometric indices (lesion width, lesion length, osteoid surface and osteoclast index) were measured using a computer program (ISSA, VAMS, Croatia). Results were statistically analyzed using Mann-Whitney *U* test.

**Results** The difference between groups in terms of lesion width was not statistically significant (group 1: 2.76 mm; group 2: 2.67 mm;  $P > 0.05$ ). The differences in lesion length (group 1: 0.79 mm; group 2: 1.20 mm), osteoid surface (group 1: 7%; group 2: 30%) and osteoclast index (group 1:  $75.96 \text{ mm}^{-2}$ ; group 2:  $5.35 \text{ mm}^{-2}$ ) were statistically significant ( $P < 0.05$ ).

**Conclusions** Electronically controlled periapical instrumentation resulted in enlarged periapical lesion (demonstrated by greater lesion length). After a 35-day healing period enhanced healing potential was demonstrated by lower bone resorption activity (e.g. greater osteoid surface and lower osteoclast index), in the electronically controlled periapical instrument group.

## R66

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### Periapical central giant cell granuloma: clinicopathologic study of four cases

**Aim** To ascertain the clinicopathologic features of periapically located Central Giant Cell Granulomas (CGCG) that were misdiagnosed as endodontic lesions.

**Methodology** Clinical and histopathologic data of biopsy specimens diagnosed as CGCGs over the past eight years were collected from the archives of the Oral and Maxillofacial Histopathology Laboratory, Geneva University, and were reviewed.

**Results** Four cases of periapical CGCG were found to be submitted with a clinical diagnosis of either radicular or residual cyst. In one case root canal treatment had been performed previously. The patients were two women and two men (three European, one North African). The age ranged from 31 to 85 years (mean age 59.2). Two cases were located in the mandibular premolar-molar region: one on the right side (from tooth 44 to tooth 46) and one on the left side (from tooth 33 to tooth 35). Two cases were situated in anteriorlateral region of the maxilla (one from tooth 11 to tooth 13, one from tooth 22 to tooth 23). Two lesions were submitted with a diagnosis of radicular cyst whereas the other two were submitted with a diagnosis of residual cyst.

**Conclusions** These data suggest that periapical CGCG may be misdiagnosed as an endodontic lesion due to its radiographic similarity to a routine inflammatory periradicular lesion. Post-treatment follow-up and routine submission of periapical surgical specimens are mandatory in order to avoid delay in the diagnosis and to perform appropriate treatment.

## R67

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### Investigation of the involvement of several non-collagenous proteins in the metabolism of human odontoblast-like cells

**Aim** To analyze proteins, known to be involved in the anabolic hard tissue metabolism of bone, in human odontoblast like (hol) cells over the course of six weeks.

**Methodology** Primary cell cultures were set up from caries free impacted wisdom teeth from donors no older than 18 years. They were pre-cultivated in an incubator up to transfer phase (T)2. In T3 they were seeded on a resin support material (Thermanox, Nunc, Germany) and transferred to a perfusion cell culture system (Minucells and Minutissue, Germany). Sampling of the hol cells was completed weekly over the course of six weeks. Analysis was made by quantitative RT-PCR (iCycler, BioRad, Germany). Different, non-collagenous proteins known to be involved in the anabolic hard tissue metabolism were quantitatively determined. These are osteonectin (SPARC), osteocalcin (BGLAP), osteopontin (SPP1), alkaline phosphatase (ALPL), fibronectin 1 (FN1) and dentinsialophosphoprotein (DSPP).

**Results** An expression pattern for hol cells was identified showing an increased expression of the dentine specific DSPP in the course of cultivation. Human pulp derived cells differentiated into odontoblast-like cells in the perfusion cell culture system and expressed dentine specific, non-collagenous proteins.

**Conclusions** The experimental model was established to compare expression patterns of odontoblast-like cells with those of anabolic hard tissue metabolism of bone to allow differences and similarities of the mineralization processes to be identified.

**Acknowledgement** The DFG (German Research Foundation) for supporting our work (RE 1562/1-2) as a member of the DFG-priority programme SPP 1117 'Principles of Biomineralization'.

## R68

## R69

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### Development of a protocol for *in vitro* culture of pulpal cells

**Aim** To apply previously established protocols for cell culture to human pulpal cells and to optimise them; and to use Relative Quantification-Real time PCR to detect and quantify the expression of dentine sialophosphoprotein (DSPP) by the cultured cells, over time.

**Methodology** Pulp tissue from 12 human extracted third molars was removed and cultured *in vitro*. The protocol for culture was modified until cell growth was obtained and confirmed by microscopy. After sub-culturing to 100%

confluence at 2, 4, 6 and 8 days, the RNA was isolated from a sample of the cultured cells from two pulp explants. RT-PCR was used to make complementary DNA and Relative Quantification-Real time PCR was used to quantify DSPP expression at the different time points relative to the expression of the 18S gene.

**Results** The initial protocols resulted in fungal and bacterial contamination or lack of adherence of the initial explant. Three of the twelve pulp tissue explants were eventually successfully and predictably cultured. A protocol to culture human pulpal cells has been proposed. Histologically, the cultured cells resembled pulpal fibroblasts. Relative Quanti-

fication-Real time PCR confirmed the expression of DSPP by the pulpal cells. There was no significant difference in the levels of DSPP expression after 2, 4, 6 and 8 days of culture.

**Conclusions** It was possible to culture human pulpal (fibroblast-like) cells using a modified protocol. Expression of DSPP by the cultured pulpal cells was confirmed but no change in expression levels was detected over 6 days in culture. A base-line model has been proposed for testing inductive changes in the cultured cells to better understand their function.

## Research Posters – Cytotoxicity and Biocompatibility

### R70

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#### *In vitro* cytotoxicity of root canal sealers

**Aim** To study cytotoxicity of the root canal sealers Diaket (3 M ESPE, Germany), IRM (Dentsply, USA), SuperEBA (Bosworth Company, USA), Hermetic (Pharma GmbH + Co.KG, Germany) and GuttaFlow (Roeko, Coltene/Whaledent GmbH + Co.KG, Germany) on human laryngeal carcinoma cells (HEP) *in vitro*.

**Methodology** The sealers were prepared according to the manufacturer's instructions under aseptic conditions and a 0.02 mL increment of each sealer was placed at the bottom of a 24-well plate. The sealers were covered with a  $1 \times 10^4$  HEP cell suspension for 1 h, 24 h, 48 h, 7 days and 1 month after mixing. Four samples and respective controls without sealer were prepared. After 5 days of incubation the number of cells was determined using an electronic counter; the number of viable cells was determined under light microscopy following the addition of methylene blue. The number of cells in the experimental dishes was calculated as a percentage of the controls.

**Results** GuttaFlow showed increased cytotoxicity over time after mixing. After one month, cytotoxicity was significantly higher (22%) than after 1 h (80%), 24 h (68%), 48 h (66%) and 7 days (50%) ( $P < 0.01$ ). Other tested materials showed strong cytotoxic effects. There were no viable cells for all tested periods.

**Conclusions** All tested materials except GuttaFlow showed strong cytotoxic effect at all periods but cytotoxicity for GuttaFlow was dependent on time.

### R71

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#### *In vitro* cytotoxicity of an antibacterial selfetch adhesive system containing MDPB

**Aim** To evaluate the cytotoxic potential of a 12-methacryloyloxydodecylpyridium bromide (MDPB) – containing antibacterial self-etching primer (Clearfil Protect Bonds, Kuraray, Japan) on L-929 fibroblasts.

**Methodology** The primer and adhesive components of the test material was diluted serially with the culture medium at a ratio of 1: 1000 and 1: 4000 (v/v). Cytotoxicity was identified by plating the L929 cell lines in 24-well culture plates in Dulbecco's modified Eagle's Medium supplemented with 10% foetal calf serum, penicillin (100 U/ml) and streptomycin at 37°C. After 24 h, the medium was changed with fresh medium containing different dilutions of the primer or adhesive components of the test material. Cells were further maintained for five days. A two-step self-etch primer/ adhesive system (FL Bond, Shofu, Japan) was utilized for comparisons. Changes in cell number within each test group was assessed statistically using Friedman Test. Inter-material comparisons were made with Kruskal-Wallis Test ( $P = 0.05$ ).

**Results** Following exposure to both dilutions of primer, the number of cells did not differ significantly within time, although the total cell number was markedly lower than that of the control group ( $P < 0.05$ ). Compared to cells exposed to 1: 4000 (v/v) of primer, some of which showed rounding or slight divergence from normal cell morphology, a marked cellular degeneration and rounded cells were observed at lower dilution factor, indicating strong toxicity. Similar to the primer, the total cell number in the adhesive was lower than that of the control group. At 1: 1000 (v/v) dilution, cells displayed more cellular alterations than those exposed to higher dilution, including nuclear condensation and cell degeneration.

**Conclusions** The primer of the tested antibacterial bonding system displayed less cytotoxicity than its adhesive component, while a higher dilution factor resulted in reduced cytotoxic effects.

## R72

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### Effect of root canal dressing on the regeneration of inflamed periapical tissue

**Aim** To evaluate the effects of an intra-canal application of chlorhexidine and calcium hydroxide on apical periodontitis in rats.

**Methodology** An experimentally induced apical periodontitis was established on the mesial roots of maxillary molars of Wistar rats by exposing the root canals to the oral cavity for 14 days. In the positive control group ( $n = 10$  teeth) the root canals received no further treatment other than the coronal access cavities were filled with composite. In the negative control group ( $n = 10$  teeth) partial pulpotomies were performed aseptically and the access cavities immediately sealed. In a third control group ( $n = 10$  teeth) the canals were instrumented, left empty and the access cavities were sealed. In the experimental groups the root canals were instrumented and either filled with 2% chlorhexidine gel (Speiko, Münster, Germany) or calcium hydroxide paste freshly prepared from pure calcium hydroxide (Merck, Darmstadt, Germany) ( $n = 10$  teeth per group). After 7 days ( $= 21$  days after initial canal exposure) all rats were killed and the histological sections were stained for microscopic analysis of periapical regeneration. The data of the subjective evaluation were analyzed with the Kruskal-Wallis test. Lesion sizes were measured and statistically analyzed using the ANOVA and post-hoc Scheffé test.

**Results** The two treatment groups showed significantly lower average inflammatory scores and smaller lesion sizes than the positive control group and the third control group ( $P < 0.05$ ). No statistically significant differences were observed between the two treatment groups ( $P > 0.05$ ).

**Conclusions** Chlorhexidine used as an intracanal medication showed good periapical regeneration, suggesting that it may be an alternative to calcium hydroxide.

## R73

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### In vitro evaluation of the biocompatible properties of mineral trioxide aggregate (MTA) as a root-end filling material

**Aim** To evaluate and compare the biocompatible property of root-end filling materials, MTA and IRM, using the osteoblast-like cell culture.

**Methodology** Cell suspension (murine MC3T3-E1 osteoblast) was added carefully over the material disks, which had been

set for 2 days at 37°C. They were incubated for 3 weeks with  $\alpha$ -MEM containing 10% FBS, ascorbic acid, and  $\beta$ -glycerophosphate. After 1 day and 2 weeks, the adhering cell number on the materials was examined using Hoechst 33 258 staining under a fluorescence microscope. Furthermore, areas in contact with the material surface were observed under a transmission electron microscope.

**Results** The number of cells adhering on MTA was significantly higher than that on IRM. In addition, ultrastructural analysis revealed numerous collagen fibrils with mineralization directly attached to the surface layer of MTA.

**Conclusions** MTA possesses better cytocompatible property than IRM.

## R74

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### Comparison of biocompatibility and cytotoxicity of two new root canal sealers

**Aim** To investigate, remote organ toxicity and connective tissue reaction of two new root canal sealers (GuttaFill and EndoRez) and compare with Kerr sealer using some biochemical and histopathological parameters.

**Methodology** Sixty white Wistar-Albino rats (weighing 200–250 g) were used. A total of 0.1 mL of GuttaFill (Roeko, Germany), EndoRez (Ultradent Products, USA) or Kerr sealer (Kerr Romulus, USA) were administered subcutaneously into the dorsal thoracic middle of rats (15 of each). Control rats were given saline only. Rats were decapitated at 24 h, on day 7 and on day 30 of the experiment and the tissue samples from lung, liver, kidney and skin were removed for the determination of malondialdehyde (MDA) and glutathione (GSH) levels. Tissues were also examined histologically. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels, and creatinine, urea concentrations (BUN) were determined to assess liver and kidney functions, respectively. Tumor necrosis factor (TNF) and lactate dehydrogenase (LDH) were also assayed in serum samples.

**Results** No statistically difference was found among the control and EndoRez, GuttaFill and Kerr root canal sealers regarding tissue MDA, GSH levels or serum parameters ( $P > 0.05$ ) at all times.

**Conclusions** Both new root canal sealers showed good compatibility and acceptable tissue toxicity.

**R75**

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### The additive effect of rhBMP-2 on the cytotoxicity of MTA cement

**Aim** To compare the cytotoxicity of MTA (Mineral Trioxide Aggregate) cement with combination treatment of MTA and BMP-2 (Bone Morphogenetic Protein-2) in MTT assays of MG-63 human osteosarcoma cells.

**Methodology** MG-63 human osteosarcoma cell line (ATCC CRL-1427, Rockville, MD, USA) was cultured in MEM media supplemented with foetal bovine serum, antibiotic-antimycotics, sodium pyruvate, nonessential amino acids, sodium bicarbonate in culture dish. MTA cement (0.2 g, Dentsply Tulsa, USA) was mixed according to manufacturer's instructions with or without one microgram of rhBMP-2 (R&D Systems, Minneapolis, MN, USA). After 1, 24, 48, and 72 h of setting time, each group of 15 samples was incubated with 2 mL culture media at 37°C, in 5% CO<sub>2</sub> and 95% humidity for

24 h. Confluent cells were counted with a haematocytometer ( $2.5 \times 10^5$  cells/ml), seeded 96-well microplate and incubated with the supernatant of each sample for 24 h. Mitochondrial dehydrogenase activity of the MG-63 cells was determined using MTT (Sigma, St Louis, MO, USA), which was converted to blue water-insoluble product formazan that accumulated in the cytoplasm of viable cells. Each group of cells was treated with MTT solution for 4 h. The absorbance of formazan was measured at 540 nm with a spectrophotometer (Specgene, Techné, France). The MTT assay was performed in triplicate and repeated in six cultures. Data were statistically analyzed with Mann-Whitney *U* test ( $P < 0.05$ ).

**Results** The scores of MTT assay increased, and the cytotoxicity of all samples decreased time-dependently. In the early period, the MTA and BMP-2 group was less cytotoxic than the MTA group until 24 h ( $P < 0.05$ ). After 48 h, the difference was not statistically significant ( $P > 0.05$ ).

**Conclusions** The cytotoxicity of MTA and combination treatment of MTA and BMP-2 decreased with time. The addition of BMP-2 had a beneficial effect that reduced initial cytotoxicity of freshly mixed MTA cement.

## Research Posters – Clinical Science

**R76**

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### Apical status of root filled teeth in a German population

**Aim** To evaluate radiologically the apical status of root filled teeth in a population in Berlin, Germany using a cross-sectional study design.

**Methodology** A total of 747 panoramic images taken between 1999 and 2001 were evaluated by one observer in a darkened room on an illuminated screen. Prior to evaluation the observer was calibrated and the intraobserver reliability was tested. Numbers of root filled teeth, apical lucencies, quality and length of root fillings were registered. Sound teeth without root fillings served as control for the judgement of apical lucencies. Relations between the apical status and the quality of root fillings as well as the quality and presence of the coronal seal were statistically evaluated using the chi-square test.

**Results** In 747 panoramic images, 17 648 teeth could be evaluated. Regardless of their filling status (root canal filling and/or coronal restoration) 6.3% of all teeth had apical lucencies. Overall, 5.6% of all examined teeth were root filled and 48.5% of these were associated with apical rarefactions. For teeth with unacceptable root fillings, chi-square test showed significantly more apical lucencies compared to teeth

with acceptable root fillings ( $P < 0.001$ ). When crowns or fillings were present as opposed to no coronal seal, significantly less apical lucencies were associated with root filled teeth ( $P < 0.001$ ).

**Conclusions** The presence of coronal restorations and an acceptable quality of root filling had a positive impact on the apical status of root filled teeth.

**R77**

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### Comparison of periapical health in a Belgian population between the periods 1992/93 and 2002/03

**Aim** To collect and compare data on the prevalence and the technical standard of root canal treatment as well as the prevalence of apical periodontitis (AP) over a 10-year time period in a Belgian population.

**Methodology** The patient records of Belgian adults attending the Dental School of the Ghent University Hospital from November 1992 to October 1993 (1) & from November 2002 to October 2003 (2) were selected. A total of 249 (1) and 681 (2) panoramic radiographs were selected and examined for endodontic treatment, periapical condition and coronal restorations.

**Results** Of the 5721 (1) and 16 229 (2) teeth examined, 6% were root filled. There were statistically significantly less teeth missing in the later cohort (2): 15% (2) versus 18% (1) ( $P < 0.0005$ ). Periapical radiolucencies were found in 6% (1) and 5% (2) of all teeth ( $P = 0.002$ ), in 44% (1) and 35% (2) of the endodontically treated teeth ( $P = 0.005$ ), and in 4% (1) and 3% (2) of non-root filled teeth ( $P = 0.003$ ). The apical level of the root fillings was inadequate in 57% (1) and 39% (2) of the root filled teeth ( $P < 0.0005$ ). There was no statistically significant difference in prevalence of AP in relation to the length of the root fillings between (1) and (2). However, when no AP was present in root filled teeth, a statistically significant increase in quality of root fillings was noted ( $P = 0.004$ ). No statistically significant differences were found between the number of teeth restored with plastic coronal fillings or crowns, and the number of teeth with caries.

**Conclusions** Although there was a statistically significant (i) increase in quality of root fillings scored on a radiographic basis and (ii) decrease of the percentage of AP on root filled teeth over this 10-year time period, the technical standard of root canal treatment remained disappointing.

## R78

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### Frequency and distribution of root filled teeth and apical periodontitis in an adult urban Croatian population

**Aim** To investigate the prevalence and quality of root fillings in teeth and the presence of apical periodontitis in an adult Croatian urban population.

**Methodology** Radiographs of 675 patients presenting for the first time at four different dental offices in Zagreb were examined. The occurrence and technical quality of root fillings were assessed for each root according to the position and the density. The periapical status was evaluated using the Periapical Index Scoring System. The data were analyzed statistically and differences were tested by ANOVA and Scheffé Post Hoc test.

**Results** A total of 17 983 teeth were examined: of these 1017 (5.6%) were root filled and 1449 (8.0%) had apical periodontitis ( $\text{PAI} \geq 3$ ). Molars were the most commonly root filled teeth (424–41.7%), then premolars (355–34.9%) and anterior teeth (237–23.5%). Root fillings ending more than five mm from the radiographic apex were observed in 530 (21.6%) roots. Root fillings ending 2–5 mm short were present in 726 (29.5%) roots. Root fillings ending <2 mm from the apex were found in 838 (34.0%) roots. Root filling material beyond the apex was noticed in 96 (3.9%) roots, and 269 (10.9%) roots had radiopaque material in the pulp chamber and/or orifice of the root canal. Adequate filling with a homogenous radiopaque material in the root canal without voids between material and walls of canal was observed in 882 (35.8%) roots. There was no difference in the frequency of endodontically treated teeth by gender or age.

**Conclusions** There was a high prevalence of periapical periodontitis and with only 34.0% root fillings ending <2 mm from apex. Overall, 35.8% well obturated root fillings were observed.

## R79

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### An assessment of periapical status in a group of Turkish patients

**Aim** To assess the prevalence and severity of apical periodontitis (AP) using the Periapical Index (PAI) and the quality of root fillings.

**Methodology** A total of 171 randomly selected male and female patients older than 13 years with mature apices having a total of 4107 teeth were evaluated clinically and radiographically, in Hacettepe University, Faculty of Dentistry, Departments of Endodontics, Ankara-Turkey. PAI scores, missing and decayed teeth, presence and standard of root fillings of the patients were recorded. The criteria to evaluate the root canal fillings were: uniformity, well condensed, apical level and were evaluated by two observers. The reliability between the observers was tested with Cohen's Kappa.

**Results** In all 130 (76.02%) of 171 patients had a total of 327 carious lesions, 106 (61.98%) of 171 patients had periapical lesions. Four (2.33%) of the 171 patients had a DMFT index score of zero. Only 6 (8.95%) of 67 root fillings were acceptable; 42 (62.68%) root filled teeth had chronic periapical lesions. Overall 106 of 4107 teeth (2.58%) had apical periodontitis.

**Conclusions** The majority of root fillings were unacceptable in terms of technical quality. The percentage of teeth with AP was high.

## R80

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### Short-term calcium hydroxide medication of teeth with large periapical lesions

**Aim** To evaluate the outcome of root canal treatment in teeth with large periapical lesions following short-term application of calcium hydroxide dressings.

**Methodology** Thirty seven teeth with a chronic or aggravated chronic periapical pathosis were selected in 34 patients (17 females and 17 males) whose age ranged from 19 to 58 years (mean  $36.4 \pm 11.6$  SD). Root canal treatment of large periapical lesions with a radiographic diameter of at least 8 mm was carried out. In each case an approximate area of the lesion was determined. The lesions whose approximate area did not exceed  $100 \text{ mm}^2$ , and those with an area of  $100 \text{ mm}^2$  or more were identified. After chemomechanical preparation of the root canals an antiseptic calcium hydroxide dressing was applied for 1–2 weeks. Finally, the canals were

filled with gutta-percha using the lateral condensation technique. The results of treatment were monitored clinically and radiographically after periods of 6–8 months, 12–14 months, 2 years and beyond. While evaluating the results of the treatment the following criteria were considered: complete healing, significant improvement, lack of improvement or exacerbation. Statistical analysis was performed using Fisher's exact test ( $P = 0.05$ ).

**Results** Thirty-four teeth out of 37 were included (91%). Complete healing was achieved in 22 cases (64%), and marked improvement in 9 cases (26%). In 3 teeth (9%) exacerbation was observed. No statistically significant difference ( $P = 0.999$ ) in successful root canal treatment between the lesions with the size  $<100 \text{ mm}^2$  (17 cases), and the lesions with  $>100 \text{ mm}^2$  (14 cases) was found.

**Conclusions** Regardless of the size of periapical pathosis conventional treatment should be undertaken. Large periapical pathosis can be treated with a short-term application of calcium hydroxide.

## R81

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### Management of apical periodontitis by Flemish general practitioners

**Aim** To gather information on general practitioners' (GPs) attitude towards treatment of apical periodontitis (AP).

**Methodology** In all 282 dentists attending peer review sessions organised by the Flemish universities were asked to complete an anonymous questionnaire in a multiple choice format. Data were entered in a database and subjected to descriptive and analytical statistics.

**Results** Five questionnaires were discarded because respondents were not general practitioners. Considering their outlook on a number of issues regarding AP, 32% of the respondents believed that the size of the radiolucency influenced the choice between conventional or surgical endodontic treatment. According to 29% of respondents, the distinction between a granuloma and a cyst can be made radiographically. Overall, 57% believed that healing of cysts is possible after root canal treatment. In relation to the endpoint of canal preparation, 38% made a distinction between vital and periapically involved teeth. Sixty percent of GPs, choose 'open drainage' as a treatment mode under certain circumstances. Regarding treatment of periapically involved teeth, the choice between single or multi-visit treatment seemed to be influenced mainly by the presence of symptoms and, in absence of symptoms, by tooth type. In case of root filled teeth presenting with AP, retreatment + follow-up was the most popular option if primary root canal treatment had been completed on a periapically sound tooth. If the tooth originally had AP, a surgical approach was more frequently chosen. When rating the type of teeth scheduled for periapical surgery 38% of respondents never apicectomized teeth, and 65% of respondents surgically treated 1–5% of root filled anterior and premolar teeth.

**Conclusions** The views and strategies of this group of GPs concerning periapically involved teeth were not always consistent with present-day recommendations.

## R82

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### The relation between apical periodontitis and root filled teeth in patients with periodontal treatment need

**Aim** To investigate factors associated with apical periodontitis in root filled teeth in a population of periodontally compromised patients.

**Methodology** The present investigation was a retrospective cross-sectional study on data collected from periodontal examination and the associated conventional intra-oral radiographic evaluation from patients attending the Department of Periodontology of the Dental School of the Ghent University Hospital. Periodontal parameters (mean probing depth, deepest pocket in relation to the cemento-enamel junction (CEJ), distance between the CEJ and the lowest marginal bone level), root canal treatment (length, homogeneity and overall quality of the root filling), the quality of coronal restorations and the history of periodontal treatment were related to the prevalence of apical periodontitis. A total of 272 root filled teeth in 94 patients were evaluated.

**Results** The periapical condition was significantly influenced by the quality of the root filling and the coronal filling ( $P < 0.05$ ). More apical periodontitis was seen when the coronal level of the root filling exceeded the marginal bone level ( $P < 0.005$ ). The marginal periodontal condition seemed to influence the periapical status. Teeth with apical periodontitis were associated with a significant increase of average probing depth ( $P < 0.001$ ), more marginal bone loss ( $P < 0.001$ ) and a higher measure of deepest pocket depth ( $P < 0.001$ ). Statistically significantly less apical periodontitis was seen in patients that had received marginal periodontal treatment ( $P < 0.001$ ).

**Conclusions** Periodontal disease may influence the periapical condition of root filled teeth. Efforts should be taken in preventing spread of infection through the periodontal-endodontic pathway by periodontal treatment and a high quality of root filling and coronal restoration. Care should also be taken to seal the coronal cavity up to the level of the root filling, where it should be advocated to reduce the coronal level of the root filling below or at least at the level of the surrounding marginal bone.



**R83**

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### Evaluation of a system for grading the complexity of root canal treatment

**Aim** To evaluate a system of grading the complexity of root canal treatment and apply it to endodontic referrals.

**Methodology** A system of grading the complexity of root canal treatment that had been previously developed was applied to all endodontic referrals to a Department of Restorative Dentistry in a District General Hospital within a period of one year. Grading was repeated in 60 randomly selected teeth to test for intra-observer and inter-observer agreement. The appropriateness of referrals and treatment undertaken was assessed in terms of the complexity.

**Results** In all 152 patients were referred for root canal treatment of 186 teeth within this period. Of these, 60 teeth were treated in the Department. There was moderate intra-observer agreement and moderate to poor inter-observer agreement with regards to the complexity grades allocated. Among the referrals, 47% (87) of teeth were of complexity grade 3 (high), 48% (89) of grade 2 (medium) and 5% (10) of grade 1 (low). Overall, 48% (29) of treatment was undertaken in teeth of complexity grade 3 and 52% (31) in teeth of complexity grade 2.

**Conclusions** This system of grading the complexity of root canal treatment was found to be simple to use, but was ambiguous and incomplete. There was moderate intra-observer agreement and moderate to poor inter-observer agreement. The referrals to the Department were of appropriate complexity.

**Acknowledgements** Mr. K. Bishop and Mr. J. Owens, Consultants in Restorative Dentistry, Morriston Hospital, Swansea; Professor R. Newcombe, Medical Statistician, Cardiff University.

**R84**

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### Endodontic surgery using endoscope visualization: 1-year follow-up

**Aim** To monitor the outcome of ultrasonic apical root-end preparation using endoscope visualization during endodontic surgery in relation to tooth type and location, and the presence of post retained restorations.

**Methodology** Teeth treated surgically had a periradicular lesion of strict endodontic origin. In all 28 anterior and premolar teeth were included in the study using specific selection criteria. A full mucoperiosteal tissue flap was reflected and carefully retracted. Surgical access to the root was then made through the cortical bone using a round bur. The periradicular lesion was removed with sharp bone curettes

and angled periodontal curettes. After exposure of the apical root-end, a straight fissure bur in a hand-piece was positioned perpendicular to the long axis of the root and then beginning from the apex, 2.5–3 mm of the root-end was removed. Prior to root-end preparation, local haemostasis was achieved through the use of bone wax. Root-end cavities were prepared using a zirconium nitrate retro-tip (Dentsply Maillefer Instruments, Switzerland) driven by an ultrasonic device unit (EMS, Switzerland). Root-end cavities were then dried using paper cones and a zinc oxide EBA-reinforced cement was used as the root-end filling material. All root-end procedures were performed using endoscope visualization. Cases were followed for a period of one year and then classified into three groups (healing, uncertain healing and disease) according to radiographic and clinical criteria.

**Results** Of the 28 teeth evaluated at 1-year follow-up, 26 teeth (93%) had healed, 1 tooth had uncertain healing and 1 had disease. There was no statistically significant differences in treatment outcome related to the type of tooth, tooth location, or presence of post restoration.

**Conclusions** In this study the adherence to a strict endodontic surgical protocol, the use of contemporary techniques and materials together with endoscope visualization lead to a predictable outcome with definite healing in 93% cases.

**R85**

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### Operating times for mechanical Ni-Ti instrumentation with two filling techniques: a preliminary report

**Aim** To evaluate the amount of time allocated to each stage of root canal treatment (total working time TWT) and to compare the time taken to complete canal filling using 2 techniques.

**Methodology** In a private clinic, general dental practitioners with varying levels of experience were instructed to recruit, on a predetermined date, patients for all types of endodontic treatment. Three independent calibrated observers carried out a double-blinded evaluation by timing each step of the consecutive endodontic treatments. After hand exploration with size 08–10 K-Flexofiles, engine-driven rotary instrumentation (ProTaper<sup>®</sup>, Dentsply-Maillefer, Switzerland) was employed for all the canal preparations. Subsequently, a similar evaluation was completed for 2 filling techniques employing thermoplasticized gutta-percha: Thermafil<sup>®</sup> system (Dentsply-Maillefer); and System B (SybronEndo, USA) with Obtura II (Obtura Corporation, USA) for vertical condensation and backfilling.

**Results** Thirteen teeth representing 35 canals were treated. Excluding radiographical examination, the mean TWT/canal was  $20.7 \pm 7.7$  mins. Seven different steps were measured: 1. Diagnosis, information and anaesthesia:  $t = 4.5$  min (range 1–11 min) 2. Access cavity preparation:  $t = 4$  min (range 1–7), exploration:  $t = 1$  min, 3. Electronic working length determination/canal:  $t = 1$  min 4. Cleaning, preparation and

drying: 5 to 7 min/canal when using a five instrument sequence (Sx-S1-S2-F1-F2) including 10–15 s for instrument changing (total = 1 min). Drying time: 1.5 min/canal 5. Thermafil® obturation/canal t = 2.5 min  $\pm$  1.7 6. Temporary filling: t = 1 min 7. Discussion t = 3 min. TWT averaged 23 min, 34 min and 44 min for single rooted, 2-rooted and 3-rooted teeth, respectively. The operating time for canal obturation with Sytem B-Obtura II was 5.9 min  $\pm$  2.4; the Thermafil® technique was faster and involved less preparation and instrumentation, without significantly influencing TWT.

**Conclusions** The major time consuming steps were canal cleaning and preparation, followed by diagnosis, anaesthesia and cavity preparation. New developments, mainly in the cleaning and preparation phase and a faster induction of anaesthesia could further shorten chair-side time.

**Acknowledgements** Jean-Emmanuel Arbez-Gindre and Gilbert Rota (Dentsply Maillefer, Switzerland) for their valuable technical support.

## R86

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### A new cone-beam computerized tomography system for use in endodontic surgery

**Aim** To present a newly developed ortho cubic super-high resolution computed tomography system (Ortho-CT) and its application in endodontic surgery.

**Methodology** A prototype was assembled on a Scanora (Soredex Findent Co, Finland) with the use of a 4-inch image intensifier instead of film. Data were collected from a single 360 degree scan and a cylinder 32 mm in height and 38 mm in diameter. Images were reconstructed with a software programme on a personal computer. Imaging data consisted of 240 (height)  $\times$  280 (diameter) cubic voxels, each with a dimension of 0.136 mm. With this small voxel size, the image resolution was high and was the same in any direction. Sections parallel to the dental arch (Parallel sections), perpendicular to the dental arch (cross sections) and horizontal sections were produced with a slice width of 1 mm at an interval of 1 mm. Patients with fractured instruments were evaluated with Ortho-CT, and the images were compared to routine radiographic films.

**Results** Ortho-CT produced images of high resolution, enabling identification of the lesions and the fractured instruments, and the relationship with the maxillary sinus and adjacent teeth.

**Conclusions** Because Ortho-CT can take high-resolution 3-dimensional images at any tomographic layer with only 1 exposure, it is a useful aid for the diagnosis and treatment of diseases in endodontic surgery.

**Acknowledgements** This study was supported by the Promotion and Mutual Aid Corporation for Private School of Japan and the Sato Fund of Nihon University School of Dentistry.